University of Alberta

The boys are back in town: *Orconectes virilis* re-introduction to Lake 302S

by

Iain David Phillips

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

in

Environmental Biology and Ecology

Department of Biological Sciences

Edmonton, Alberta

Fall 2006

Library and Archives Canada

Published Heritage Branch

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque et Archives Canada

Direction du Patrimoine de l'édition

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence
ISBN: 978-0-494-22343-7
Our file Notre référence
ISBN: 978-0-494-22343-7

NOTICE:
The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:
L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

ABSTRACT

The crayfish *Orconectes virilis* is re-colonizing lakes in eastern Canada as post-acidification recovery continues, and is expanding its range westward into the northern Great Plains. In addition, the closely related congener *O. rusticus* from the United States is expanding its range in into boreal Canadian watersheds. Here I review the distribution, invasion potential, and ecological impacts of *O. virilis* and *O. rusticus*. Using a mesocosm experiment I show that the re-introduction of *O. virilis* significantly ($p < 0.05$) suppressed zoobenthos biomass by 70% and periphyton biomass ($p < 0.001$) by 90% in a recovering acidified lake. Stable isotopes and gut analysis further show that this species is an omnivore. My findings suggest that re-introduction of *O. virilis* must be controlled carefully because in the absence of benthivorous fish *O. virilis* populations can exert pronounced negative effects on the production capacity of boreal lakes for harvestable fish.

ACKNOWLEDGEMENTS

I would like to thank Rolf Vinebrooke for his strong leadership and creative inspiration, qualities that were invaluable to the re-introduction experiment and supportive lab environment at the University of Alberta.

I appreciate the professional criticisms and field resources provided by the researchers at the Experimental Lakes Area, Ontario, and the George Lake Field Station, Alberta. This field research greatly benefited from the help of Michael Turner, the Freshwater Biodiversity Laboratory, Marie-Claire St. Jacques, Patrick Thompson, and my parents in Lumsden, Saskatchewan.

Exchanges with friends in the Vinebrooke, Spence and Schindler labs over the course of my tenure at the University of Alberta were extremely valuable in shaping the ideas in this thesis. In particular, I would like to thank Mike Christensen, Mark Graham, Tyler Cobb, John Spence, and Mark Brigham (University of Regina) for their constructive criticism and expertise. Finally, a special thank you to Vanessa Phillips and my family for their love and support over the course of our time in Edmonton.

This project was funded by a Natural Sciences and Engineering Research Council of Canada Discovery grant, and an Academic Science Subvention grant from the Department of Fisheries and Oceans Canada to Rolf Vinebrooke, along with financial support from the University of Alberta.

TABLE OF CONTENTS

CHAPTER ONE **1**

INVASIONS BY *ORCONECTES VIRILIS* AND *O. RUSTICUS* IN CANADA: A REVIEW **1**

INTRODUCTION 1

Using native crayfish to help restore Canadian boreal lakes *1*

Potential invasion of Canadian boreal lakes by exotic crayfish *3*

Potential effects of exotic crayfish on native crayfish in boreal lakes *4*

BACKGROUND 6

General biogeography of O. virilis *and* O. rusticus *in Canada* *6*

General biology of O. virilis *and* O. rusticus *7*

Dynamics of invasion *8*

Reproductive habitat requirements, competition, and predation *11*

Ecosystem impacts *14*

Options for ecosystem management and mitigation of invasions *21*

THESIS OBJECTIVES 22

LITERATURE CITED 28

CHAPTER TWO **44**

RE-INTRODUCTION OF CRAYFISH TO A RECOVERING ACIDIFIED LAKE: ECOLOGICAL RESTORATION OR INVASION? **44**

INTRODUCTION 44

METHODS 47

Study site *47*

Experimental design *47*

Crayfish introductions *48*

Zoobenthos sampling 49

Periphyton 49

Leaf detritus 50

Gut-content analysis 51

Stable isotope analyses 52

Statistical analyses 53

RESULTS 54

Effects of O. virilis on zoobenthos 54

Effects of O. virilis on periphyton and leaf litter 54

Gut and stable isotopic analyses 55

DISCUSSION 56

Impacts of O. virilis on zoobenthos 57

Effects on periphyton 59

LITERATURE CITED 73

CHAPTER THREE 85

GENERAL CONCLUSIONS AND MANAGEMENT IMPLICATIONS 85

GENERAL CONTEXT 85

CONCLUSIONS 85

Areas of future research 85

Monitoring of range expansion 86

Regions of likely range expansion 87

Management implications of reintroduction 88

LITERATURE CITED 91

LIST OF TABLES

Table 1.1. Crayfish of Canada 24

Table 2.1.a. Pooled taxa list of invertebrates collected by abundance, biomass and treatment. Trophic relationship codes: P = predator, C = collector, G = gatherer, F = filterer, S = shredder. 61

Table 2.1.b. Table 2.1.a continued 62

Table 2.2. Repeated Measures ANOVA results for the negative effects of crayfish introductions on periphyton and benthic invertebrate biomasses. Values are *F* statistics for n=5 replicates. **p*<0.05, ***p*<0.001..... 63

Table 2.3. Repeated Measures ANOVA results showing the negative effects of crayfish introductions on pigment concentrations. Values are F statistics for n=5 replicates. *p<0.05, **p<0.001..... 64

LIST OF FIGURES

Figure 1.1. Approximate distribution of *Orconectes virilis* and *O. rusticus* in Canada. Adapted from Brock University Map Library. 25

Figure 1.2. Areas of range expansion for *Orconectes virilis* and *O. rusticus* in Canada. Adapted from Brock University Map Library. 26

Figure 1.3. Hypothesized impacts of re-introduced (*Orconectes virilis*) and subsequent invasion by exotic (*Orconectes rusticus*) crayfish on littoral food webs in boreal lakes. — strong interaction. — moderate interaction. --- weak interaction, and --- in both directions indicates competition. PRED. INVERTS. = Predatory Invertebrates. HERB. INVERTS. = Herbivorous Invertebrates. 27

Figure 2.1. Hypothesized direct and indirect effects of omnivorous *Orconectes virilis* on periphytic algae upon reintroduction to post-acidification lakes. In post-acidification lakes (left) devoid of the natural *O. virilis* population, large predatory invertebrates control the abundance of other invertebrate predators, and herbivorous invertebrates to release periphyton from grazing pressure. The reintroduction of extirpated *O. virilis*, will result in significant top-down suppression of large invertebrate predators, however this will not translate into a trophic-cascade as *O. virilis* will exert direct predation on smaller invertebrates and grazing pressure on periphyton as well..... 65

Figure 2.2. Mean (±1 SE) biomass of total benthic invertebrates in *O. virilis* enclosures, and control enclosures in L302S from June to August 2004. 66

Figure 2.3. Mean (±1 SE) biomass of benthic invertebrates in *O. virilis* enclosures, and control enclosures in L302S from June to August 2004. 67

Figure 2.4. Mean (±1 SE) abundance of the dominant benthic predator *Aeshna* in *O. virilis* enclosures, and control enclosures in L302S from June to August 2004. 68

Figure 2.5. Mean (±1 SE) biomass of periphyton in *O. virilis* enclosures, and control enclosures in L302S from June to August 2004..... 69

Figure 2.6. Mean (±1 SE) chl *a* pigment concentration per unit dry weight from tiles in *O. virilis* enclosures, and control enclosures in L302S from June to August 2004. 70

Figure 2.7. Mean (±1 SE) concentration of algal pigments in *O. virilis* enclosures, and control enclosures in L302S from June to August 2004. 71

Figure 2.8. Results of stable isotope analyses showing mean (±1 SE) $\delta^{15}N$ and $\delta^{13}C$ of benthic fauna from L302S over summer 2004. Replicates used in analysis in parentheses. 72

CHAPTER ONE

INVASIONS BY ORCONECTES VIRILIS AND O. RUSTICUS IN CANADA: A REVIEW

INTRODUCTION

Crayfish are often the largest aquatic invertebrates in temperate freshwater ecosystems (Lorman 1980, Momot 1984), and have dramatic effects on their environments through feeding activities (e.g., Olsen et al. 1991). These impacts are particularly pronounced when crayfish expand their range into new water bodies that lack these aggressively feeding omnivores. Range expansions of *Orconectes* crayfish to the boreal lakes and rivers of western Canada and Ontario devoid of resident crayfish species may be particularly vulnerable to invasion as they have the physical and biotic environment compatible with these orconectid crayfish. Either as a result of anthropogenic acidification or glaciations many of these boreal lakes lack resident crayfish species to buffer against invasions, and lakes in western Canada lack important predators of crayfish that might otherwise slow or inhibit invasion (M.G. Sullivan, Department of Biological Sciences, University of Alberta, personal communication, 2006).

Using native crayfish to help restore Canadian boreal lakes

Eastern Canadian boreal lakes have experienced severe acidification as a result of industrial activities, leading to the extirpation of *Orconectes virilis* Hagen from many watersheds (Heneberry et al. 1992). Acid sensitive boreal lakes in the Sudbury region of Northern Ontario experienced severe declines in pH due to heavy

industrial metal smelting through the mid-20th century. As pH returns to natural levels, many previously native taxa are conspicuously absent, but once held important food-web positions in pre-acidified water bodies (Keller et al. 1999). Currently, the absence of species such as *O. virilis* has allowed affected lakes to recover to different states than had existed prior to the severe perturbation (Keller et al. 1999). As these boreal lakes recover from acidification, re-colonization will inevitably occur through connecting water pathways to watersheds that still possess crayfish assemblages. The effect of re-colonization may resemble that of an invasion involved with *O. virilis* range expansion as the lakes have established new benthic communities in the absence of crayfish since extirpation due to anthropogenic acidification.

It may be necessary to use biomanipulation in the form of controlled reintroductions to restore the natural faunal communities of these lakes and the ecosystem processes with which they were associated. For example, the omnivorous copepod *Hesperodiaptomus arcticus* Marsh was re-introduced into Canadian alpine lakes after removal of previously stocked fish to restore the lakes to their natural fishless state (McNaught et al. 1999). Further, extirpations of native crayfish (*Astacus astacus* Linnaeus) in Europe led to ecosystem management strategies involving successful reintroductions of these crayfish to their native water bodies (Taugbøl and Peay 2004). Using native crayfish such as *O. virilis* to restore the natural ecosystem processes in boreal lakes may be an important step in the mitigation of the effects of earlier anthropogenic perturbations such as acidification.

Potential invasion of Canadian boreal lakes by exotic crayfish

The rusty crayfish (*Orconectes rusticus* [Girard]) is currently expanding its range in Canada (Crocker and Barr 1968, Berrill 1978, Momot 1992). *O. rusticus* is an exotic crayfish introduced from the United States (US) currently expanding its range in Ontario (Momot 1995). Invasions of this species have led to dramatic changes in certain ecosystems (e.g., Lodge and Lorman 1987, Olsen et al. 1991, Lodge et al. 1994, Wilson et al. 2004, McCarthy et al. 2006). The US has experienced a dramatic range expansion of *O. rusticus* over the past half century, and experience gained there may be helpful in understanding current range expansions in Canada. Originally described from the Ohio River at Cincinnati, *O. rusticus* is thought to be endemic to the Ohio River and its tributaries in Ohio, northern Kentucky, and Indiana (Creaser 1931, Page 1985). Through introduction largely attributable to its use as fishing bait (Hobbs III et al. 1989, Page 1985) the species is known to have expanded its range to at least half a dozen other states (Taylor and Redmer 1996), and to Ontario here in Canada (Crocker and Barr 1968) (Fig. 1.1).

It is commonly held that invaders are especially successful when their particular niche is unoccupied in the ecosystem being invaded (see Lodge 1993*a*). However, because *O. rusticus* is quite proficient at displacing native congeners (Momot et al. 1978, Capelli and Munjal 1982), this species does not need an open niche to invade boreal or temperate lakes. In northern Wisconsin lakes, *O. rusticus* replaced two resident orconectid crayfish species (including *O. virilis*) to dominate the benthic crayfish fauna (Hill et al. 1993). These observations suggest that even

establishment in ecosystems by the native Canadian orconectid fauna will not act as a buffer against prolific range expansion of *O. rusticus* as suggested by Crocker and Barr (1968).

Potential effects of exotic crayfish on native crayfish in boreal lakes

The threat of exotic species toward native biodiversity is a global problem (Lodge 1993*b*, Vitousek et al. 1996, Williamson and Fitter 1996, Hill and Lodge 1999, Lodge et al. 2000). In North America conservationists have identified exotic species as responsible for the extinction or endangerment of many native fish species (Miller et al. 1989) and more recently of freshwater invertebrates (Dobson et al. 1997). *O. rusticus* has a dramatic ecosystem effect, dominating that of *O. virilis* (Fig. 1.2). *O. rusticus* has a higher metabolic rate and larger appetite than its congeners (Jones and Momot 1983), thus leading to dramatic reductions in benthic macroinvertebrates (Olsen et al. 1991, Momot 1992, Lodge et al. 1994) and macrophytes (Lodge and Lorman 1987). It is the magnitude of macrophyte disturbance that is the most notable difference between *O. rusticus* and *O. virilis* (Fig. 1.2). Although *O. virilis* reduces macrophyte abundance (Chambers et al. 1990), *O. rusticus* more effectively eliminates whole macrophyte beds as is evident by their impact on aquatic plants in lakes that already possess *O. virilis* (Wilson et al. 2004). Such large-scale macrophyte removal may also result indirectly in large reductions of macroinvertebrates, changes to fish habitat, and may even destabilize the aquatic ecosystem.

Of Canada's 11 crayfish species *Orconectes virilis* is the most widespread;

occurring in more provinces than all other crayfish species combined (Table 1.1). Here I focus on *O. virilis* because of its wide distribution. No other crayfish species in Canada is on the fringe of so many uninhabited aquatic habitats. Understanding the impact this species will have on ecosystems it colonizes, and the invasion pathways that facilitate colonization are important to determine if this species needs to be controlled for potential negative impacts it may have.

Further, of the remaining crayfish species, *O. rusticus* has the greatest invasion potential. Although the Signal crayfish (*Pacifastacus leniusculus*) has been shown to have a dramatic effect as an invader on native crayfish and benthic invertebrate fauna in other areas of the world (Nyström et al. 1999, Lodge et al. 2000), it is limited in Canada by the continental divide. The potential of its expansion east of British Columbia is not great unless it receives significant human assistance. Therefore, the aquatic corridors available where *O. rusticus* has established in Ontario (Fig. 1.1) present the next greatest potential risk for crayfish range expansion. This is of particular concern due to the associated devastating impacts it wreaks on the aquatic ecosystems it invades (e.g., Lodge and Lorman1987, Olsen et al. 1991, Lodge et al. 1994, McCarthy et al. 2006).

In this chapter I review the current documented distribution of these species in Canada, and identify the potential impacts further range expansions may have on boreal aquatic ecosystems.

BACKGROUND

General biogeography of O. virilis *and* O. rusticus *in Canada*

Canada has 11 species of crayfish with at least one species present in each province except Nova Scotia, Prince Edward Island, and Newfoundland (Table 1.1). *O. virilis* is the most widespread crayfish in Canada, occurring in more provinces than all the other species combined (Fig. 1.1). *O. virilis* is known to naturally occur east of the continental divide from Saskatchewan to New Brunswick (Crocker and Barr 1968, Clark and Lester 2005, McAlpine et al. 1999), but has recently been expanding into Alberta (Fig. 1.2) where its local distribution has not yet been fully documented (H. Proctor, Department of Biological Sciences, University of Alberta, personal communication). Capelli and Munjal (1982) have suggested that the distribution of *O. virilis* is a function of their post-glaciation re-colonization. With the retreat of the last glaciers approximately 10,000 years ago *O. virilis* was probably the first to colonize the new aquatic environments (Berrill 1978, Capelli and Munjal 1982). It appears that the absence of *O. virilis*, (or of any other crayfish) in some parts of western Canada is likely due to their slow natural re-colonization.

Crocker and Barr (1968) listed *O. rusticus* as being rare in Ontario relative to *O. virilis*; however, its range is expanding and is likely limited merely by its capability to disperse to uninhabited watersheds. Crocker and Barr (1968) originally recorded *O. rusticus* to be limited in Canada to the Kawartha Lakes district, and to the Lake of the Woods. This has been further clarified by Berrill (1978) who compiled a detailed documentation of the local expansion of *O. rusticus* throughout the Kawartha Lakes district, Ontario. Since the original accounts, range expansions

of *O. rusticus* have been recorded near Thunder Bay, Ontario, in the Lake Superior Basin (Momot et al. 1988, Momot 1992) (Fig.1.1).

General biology of O. virilis *and* O. rusticus

O. virilis and *O. rusticus* have life-cycles characterized by marked synchrony in events between the species (Crocker and Barr 1968). Copulation occurs in autumn and/or spring resulting in fertilization of the eggs that mature females extrude in spring and carry for a few weeks until they hatch (Berrill 1978). This stage is also described as "in berry" for the conspicuous presence of dark eggs attached to the abdomen of the female. Young crayfish remain with their mothers through instar stages I and II, but leave when they molt into stage III, thus becoming independent in early summer. Young-of-the-year crayfish will molt several more times before autumn. Some will become sexually mature before winter, but most do not mature until the spring. These orconectid crayfish males will molt out of sexually functional form I in the spring and back again from form II in middle or late summer while mature females molt only once after their offspring leave (Berrill 1978).

Crayfish have traditionally been viewed as detritivores and herbivores (e.g., Huryn and Wallace 1987, Webster and Patten 1979). However, contemporary workers such as Momot (1995) assert that the rapid growth and high survival of crayfish could not be sustained primarily through plant material and detritus, and therefore crayfish must be primarily carnivorous to meet their growth requirements. Further support for Momot's (1995) assertion can be drawn from the dramatic effect

some crayfish such as *O. virilis* and *O. rusticus* have on macroinvertebrate communities (e.g., Hanson et al. 1990 and Olsen et al. 1991 respectively). Despite the necessity of animal matter in orconectid diets (Momot 1995), they still exert strong consumptive activities on both macrophytes and detritus in boreal lakes (Jones and Momot 1983).

Population density is one of the key factors determining *O. virilis* growth (Momot et al. 1978). Momot and Gowing (1977) found that human harvesting of crayfish populations leads to an increase in average growth of the remaining crayfish, likely as a result of decreased competition, and increased food resources.

Dynamics of invasion

Presumably the most successful crayfish range expansions will occur in water bodies with the best crayfish habitat, food, reproductive requirement compatibility, and with the least amount of competition or predation. It is important to characterize what orconectid requirements are most valuable, and from what other organisms significant competition or predation may occur when projecting the possibility of future orconectid range expansions.

Little has been reported on the invasion pathways specific to *O. virilis*. However, *O. rusticus* has been studied extensively in the northern US, and invasions to new watersheds are primarily associated with human behaviour. *O. rusticus* expansion has been largely attributed to "bait-bucket introductions" (Page 1985, Taylor et al. 1996). Capelli and Magnuson (1983) revealed a strong correlation between the presence of *O. rusticus* and human use of lakes in the 1970's and

suggested that introductions by anglers is the main vector of *O. rusticus* range expansion. Crayfish are easily transported overland and can be introduced to new aquatic habitats when discarded as unused bait. The isolated nature of the Lake of the Woods region, Ontario (Fig. 1.1) relative to *O. rusticus'* native range suggests that the occurrence of the rusty crayfish there is likely the result of such an introduction. However, the occurrence of *O. rusticus* in the Thunder Bay area has been attributed to its dispersal along the coast of Lake Superior from Minnesota (Momot 1992) (Fig. 1.1).

O. rusticus has a high dispersal capability, and now that it is establishing in Canada's boreal region, has a high potential to rapidly colonize connecting watersheds. Although highly variable, it is not exceptional for *O. rusticus* to travel >200 m in 48 h (Byron and Wilson 2001). Further, Puth and Allen (2004) determined that in addition to human activity, stream connections between water bodies also played a significant role in *O. rusticus* invasion. This has important implications here in Canada where point introductions to water bodies such as Lake of the Woods may facilitate "jumps" across otherwise prohibitive watershed boundaries, and allow *O. rusticus* to spread through connecting water bodies.

O. virilis likely uses similar mechanisms of range expansion primarily facilitated through bait-bucket introductions and relatively slower dispersal through connecting water bodies. However, further investigation into differences and similarities between the two species' range expansion mechanisms is necessary to project future range expansions of these two species and areas of interaction with other crayfish species.

The most important habitat requirements for the two orconectid crayfish studied here are in the littoral zones of the lakes and streams that they may be colonizing. North American crayfish are generally grouped into two coarse behavioral classifications, that of burrowers and non-burrowers (Taylor et al. 1996). Both *O. virilis* and *O. rusticus* are non-burrowers (Momot et al. 1978, and Taylor and Redmer 1996 respectively) that typically occupy permanent waters, and spend daylight hours in hiding (except during *O. rusticus* reproduction, see below). *O. virilis* can attain large population densities in some lakes; densities are positively influenced by allochthonous fertilization and habitat availability (Momot et al. 1978). *O. virilis* commonly occurs in streams, rivers and lakes with rocky bottoms preferring habitats with a large amount of habitat structural complexity for protection from predation (Crocker and Barr 1968, Stein 1977).

Taylor and Redmer (1996) conducted a study of *O. rusticus* dispersal in Illinois and found that habitat complexity is a crucial habitat requirement. In both lakes and rivers, *O. rusticus* usually occurred in areas with rocks or fractured concrete substrate, and the authors suggest that large cobble substrate is likely the limiting factor in the distribution of *O. rusticus* in Illinois. Further, Wilson et al. (2004) found that water depth limited *O. rusticus* movement and colonization. This may be important in areas such as Lake Superior where *O. rusticus* invasion may be hampered by bays deep enough to slow the expansion of a successful breeding population as it expands along the littoral zone of the lake (Momot 1992).

If animal-based food material is insufficient, crayfish consume a wide variety of materials. Therefore, food prerequisites for crayfish invasion are likely less

important than other environmental and competitor or predation factors. However, Momot (1992) projects that the large macrophyte beds along the shallow, warm bays of the north shore of Lake Superior will facilitate further *O. rusticus* range expansion east from its current isolated occurrence at Thunder Bay. In highly connected water bodies, food availability may determine the rate of range expansion. However, the effect that local food quality has on the ability of *O. virilis* or *O. rusticus* to disperse is not as well understood (but see Momot 1995) and habitat is likely more important as a limiting factor in crayfish density and range expansion rate (Taylor and Redmer 1996).

Reproductive habitat requirements, competition, and predation

There are few specific requirements for orconectid reproductive habitat. However, the principal mating season for *O. rusticus* is during the period of seasonal cooling in September and October. Immediately following copulation, in soft-bottom ponds, female *O. rusticus* construct horizontal burrows in the banks of the water body near the water line that they will occupy through the winter (Crocker and Barr 1968). In the spring the eggs hatch, and the young-of-the-year leave the female shortly thereafter. It is possible that water level fluctuations may endanger gravid female *O. rusticus* during this time, but I could find no studies specifically addressing that possibility. Thus there are likely no specific reproduction habitats necessary for these crayfish, although the female crayfish in-berry will require refuge for every-day protection.

Competition with congeners has been suggested as a natural check for

invading *O. rusticus* populations (Crocker and Barr 1968). However, more recent research shows that congeners do not inhibit colonization; in fact, the closely related *Orconectes propinquus* (Girard) may facilitate *O. rusticus* invasions (Perry et al. 2001, 2002). Perry et al. (2001, 2002) found that *O. rusticus* can hybridize with *O. propinquus* and establish a foothold in new aquatic environments even faster by inhibiting reproduction of other native crayfish. *O. propinquus* occurs in many locations of Ontario currently subject to *O. rusticus* invasion (Crocker and Barr 1968), and may prove to be an important factor in its range expansion.

Because of the consistent success of *O. rusticus* invasions and displacement of resident crayfish species, it has received a large amount of attention in the US (Abrahamsson 1971, Capelli and Munjal 1982, Butler and Stein 1985, Lodge and Lorman 1987, Olsen et al. 1991, Garvey and Stein 1993, Hill and Lodge 1994, Lodge et al. 1994, Kershner and Lodge 1995, Wilson 2002). It also is implicated in the decline of native crayfish species here in Canada (Berrill 1978). Factors influencing the dominance of *O. rusticus* over other crayfish range from morphological and behavioral attributes of *O. rusticus* (Capelli and Munjal 1982, Garvey and Stein 1993, Garvey et al. 1994), to selective predation on its congeners resulting from a combination of these factors forcing them into higher-predation risk situations (DiDonato and Lodge 1993, Mather and Stein 1993). The more aggressive nature and larger body size of *O. rusticus* allow it to displace individuals of other species from habitat that would otherwise provide refuge and thus increase predation rates of those displaced crayfish (Taylor and Redmer 1996). Bergman and Moore (2003) found that direct aggressive interactions between *O. virilis* and *O.*

rusticus are typically dominated by *O. rusticus*, particularly when occurring near shelters or food resources. The ability of *O. rusticus* to aggressively out-compete *O. virilis* for such key resources may be important to expanding its range in boreal lakes.

In addition to abiotic habitat components, predation constrains crayfish populations. Potential crayfish predators in areas of *O. virilis* range expansion in western Canada include walleye (*Sander vitreus* [Mitchill]), northern pike (*Esox lucius* [Linnaeus]) and yellow perch (*Perca flavescens* [Mitchill]) (Nelson and Paetz 1992). Areas of *O. rusticus* range expansion in Ontario have a much more diverse crayfish-eating fish fauna, as many of these species have been documented to feed on orconectid crayfish (see Dorn and Mittelbach 1999). *Orconectes* have been found to comprise the most energetically important component in the diets centrarchids like rock bass and smallmouth bass in lotic environments (Rabeni 1992). Further, Rabeni (1992) found that these fish were responsible for having consumed nearly a third of crayfish production in a season. However, Gowing and Momot (1979) concluded that brook trout (*Salvelinus fontinalis* [Mitchill]) were not particularly good at consuming *O. virilis* as only small crayfish were eaten, and these only by large fish.

The importance of gape-size in fish predation on crayfish may reduce natural control of orconectid populations in areas of Canada that have experienced intense size-selective fishing pressure. While native walleye stocks in Alberta may have been able to act as significant crayfish predators prior to sport fishing, the mean size of the fish in these populations have decreased as a result of over-fishing (Sullivan

2003). The reduction in mean fish size will likely have decreased the number of large fish able to consume adult *O. virilis* as these crayfish invade Alberta water bodies. Further, Alberta lacks centrarchid fish populations (M.G. Sullivan, Department of Biological Sciences, University of Alberta, personal communication, 2006), which are significant potential crayfish predators. Therefore it is certainly possible that the fish assemblages on the fringe of orconectid range expansions in Canada lack the natural characteristics that would otherwise check or even slow further range expansion and mitigate the impact of such growth.

Ecosystem impacts

As the largest invertebrate omnivores in aquatic ecosystems, crayfish can exert pronounced effects on their environment. Both *O. virilis* and *O. rusticus* play important roles in trophic interactions, and function as keystone species in aquatic ecosystems (Momot et al. 1978). These crayfish feed on a wide variety of organisms, from vulnerable stages of vertebrates (e.g., fish eggs, larval amphibians) to benthic invertebrates, periphyton and detritus. As omnivores orconectid crayfish directly link both primary production- and detrital-based food webs to fish capable of eating them (see Dorn and Mittelbach 1999). Further, Momot et al. (1978) argue that omnivorous crayfish provide stability in an aquatic ecosystem, preventing any one trophic level from dominating, and thus maintain community structure.

Crayfish have been identified as ecosystem engineers (Statzner et al. 2000), which means that invasions of orconectid crayfish may lead to dramatic environmental and community changes. The most prominent abiotic impacts

crayfish typically exert on their environment is the destruction of the structural habitat complexity provided by vegetation, sediment resuspension, and bioturbation. Although *O. virilis* can significantly reduce macrophyte beds (Chambers et al. 1990), the impact of *O. rusticus* is even greater (Lodge and Lorman 1987, Olsen et al. 1991, Lodge et al. 1994). For many fish species, macrophytes are important habitat for juveniles (Mittelbach 1981), and destruction of macrophytes may be seen as ecosystem-engineering with the associated reduction in habitat complexity of littoral zones through non-consumptive means (Lawton 1994).

Aquatic invertebrates are increasingly being identified as important agents governing sediment transport in flowing waters (e.g., stoneflies [Statzner et al. 1996], blackflies [Wotton et al. 1998] and caddisflies [Statzner et al. 1999]). The invasion of orconectid crayfish into lotic environments may also affect this important geomorphic process. Vertebrates have typically been identified as agents of sediment re-suspension, such as that brought about by salmon eroding streambeds during spawning nest construction (Kondolf 2000), or during feeding by small fish (Flecker 1996). However, crayfish can also reduce silt cover on the bottom and re-suspend sediments (Parkyn et al. 1997), thus influencing egg survival of gravel-breeding fish (Statzner et al. 2000). Statzner et al. (2003) found that orconectid crayfish significantly contribute to sediment re-suspension, and can affect the patch dynamics of major sediment transport events such as floods. Therefore the invasion of *O. virilis* has the potential to affect the overall structural and functional biodiversity of lotic communities in areas of western Canada.

Crayfish have direct and indirect effects on the abundance of primary

producers in their environment. Crayfish prey on grazers, which may allow for increases in periphyton biomass. The effect of crayfish on periphyton may not be as simple though, as crayfish also feed on periphyton, and may directly offset this trophic effect through their own consumption. Further, in pursuit of higher quality prey, orconectid crayfish can physically disturb mats of periphyton with effects on the benthic community similar to direct consumption.

However, there are potential differences in the impact both species exert on the periphyton matrix. Lodge et al. (1994) found that *O. rusticus* can significantly alter the composition of a periphyton matrix through the physical disturbance of their foraging activities. *O. rusticus* displaces the detrital components of the periphyton, increasing the proportion of firmly attached chlorophyll-inferred algal biomass in the periphyton. *O. virilis* has a contrasting effect on the periphyton matrix (covered in more detail Chapter 2, Fig. 2.6) wherein there is no significant difference in the effect of this species on one component of the periphyton matrix over the other despite an overall impact on the periphyton dry mass and chlorophyll *a* (chl *a*) inferred algal biomass. Therefore, *O. virilis* likely grazes the periphyton matrix, while *O. rusticus* merely displaces the loosely attached detrital material associated with the periphyton matrix in its other activities.

The macroscopic alga *Chara* is often a desired food for *O. virilis* despite its low nutritional value relative to other macrophytes (Chambers et al. 1990). Because *O. rusticus* has such a pronounced effect on macrophyte biomass (Lodge and Lorman 1987) they will likely also reduce this alga; however, I could find no specific study involving the two organisms. As a result, reductions in the biomass of

this alga should be expected in areas *O. virilis* or *O. rusticus* are invading. Dorn and Wojdak (2004) document *O. virilis* induced reductions in green metaphyton, particularly *Chara*. Further, it has even been found that orconectid crayfish in Georgian Bay, Lake Huron prefer *Chara* to gastropods (Saffran and Barton 1993). Saffran and Barton (1993) suggest that *Chara* may be an important source of calcium that is easier to digest than other macrophytes as it lacks cellulose. Telford (1970) found that *O. virilis* lacks cellulase in its digestive enzymes, and thus would find little nutritional value in other vascular plants. This is also probably true for *O. rusticus*, although I could find no confirmatory study.

Another reason for orconectid *Chara* foraging may be indirect. It is highly likely that *Chara* serves as a substrate for other benthic macroinvertebrates and microfauna (Momot 1995). Hanson et al. (1990) supported this assertion, and found that *Chara* hosted a larger fauna than any other substrate in their Alberta ponds. Regardless, *O. virilis* and *O. rusticus* both negatively impact benthic algal communities.

Although the effect of crayfish on pelagic communities has received little attention, *O. virilis* and *O. rusticus* may affect phytoplankton by resuspension of benthic nutrients and by nutrient secretion. Dorn and Wojdak (2004) found that phytoplankton biomass was elevated in ponds containing *O. virilis* relative to those without during one year of an experiment, but that result flipped to lower phytoplankton biomass in the second year of the experiment. The authors attribute high rates of bioturbation and nutrient excretion as the cause of elevated chl *a* in the first year, and the reduction of crayfish populations as the cause of reduced impact in

the second year (Dorn and Wojdak 2004). These findings emphasize the need to measure planktonic parameters in future research with crayfish to better understand their influence on the whole ecosystem.

Both species of *Orconectes* discussed here have dramatic impacts on macrophyte communities. However, because *O. rusticus* has a higher metabolic rate (Momot 1984), larger individual size (Momot 1992), and often reaches higher population densities (Lodge et al. 1986) its effects can far exceed those of *O. virilis*.

Macrophytes can be integral components of boreal shield lakes as reviewed by Engel (1990). An important impact of their removal on the trophic structure could be an indirect effect on the associated benthic invertebrate fauna. In addition to providing nesting surfaces for sport fish (e.g., northern pike), macrophytes provide important living space for the invertebrates both forage and sport fish rely on.

Macrophytes themselves are typically considered poor food resources for crayfish. The dramatic impact of *O. virilis* and *O. rusticus* on macrophyte biomass (Chambers et al. 1990, and Lodge et al. 1994 respectively) may actually be due to mechanical removal of macrophytes during the search for associated benthic fauna. This suggestion is supported by the apparent lack of interest crayfish subsequently pay to the detached macrophytes (Momot 1995). Those macrophytes that are ingested may serve as bulk filler in the absence of better food, as a substitute for higher protein food sources, as a source of micronutrients, or even incidentally ingested in the search for other organisms (Momot 1995). Whatever the reason for grazing of macrophytes by orconectids, it can be expected that macrophyte biomass

will be reduced in newly invaded watersheds, potentially destabilizing the ecosystems.

Despite the aforementioned effect of *O. rusticus* on congeners, small-scale mesocosm studies of its impact on zoobenthos have generated incongruent results (Lodge et al. 1994, Perry et al. 2000, Charlebois and Lamberti 1996, Perry et al. 1997, Stewart et al. 1998). McCarthy et al. (2006) recently used meta-analysis of several short experimental studies along with a long-term data set time series to identify consistent patterns of *O. rusticus* effects on aquatic invertebrate abundances. They identified negative impacts on Gastropoda, Odonata, Trichoptera, Amphipoda, Ephemeroptera, and Diptera. Snails are particularly vulnerable to predation by both *O. rusticus* (Lodge and Lorman 1987, Olsen et al. 1991, Lodge et al. 1994) and *O. virilis* (Crowl and Covich 1990, Hanson et al. 1990). These results are consistent with Wilson et al. (2004) who found similar impacts of *O. rusticus* on zoobenthos in a long-term study of a lake invasion.

The community impacts of crayfish include both direct and indirect effects on the littoral zooplankton. Juvenile crayfish actively pursue and consume live *Daphnia*, and show increased growth rates when preying on zooplankton in addition to detrital and herbivorous food sources (Brown et al. 1992). These crayfish actively seized and subsequently ingested *Daphnia* with their chelae. Although filter feeding has not yet been recorded as an important mechanism in crayfish feeding on zooplankton, Momot (1995) has observed crayfish (presumably *O. virilis*) filter-ingesting self-turbated benthic material with their feeding apparatus.

However, indirect effects may confound whatever impact direct predation by

O. virilis may have on zooplankton populations. For example, Dorn and Wojdak (2004) found that zooplankton biomass increased in ponds occupied by *O. virilis* relative to controls. This was attributed the negative impacts of *O. virilis* on fish recruitment. An increase in phytoplankton due to bioturbation and nutrient excretion may also positively influence overall zooplankton productivity (Dorn and Wojdak 2004).

Although Rabeni (1992) found that orconectid crayfish account for a major component of smallmouth bass and rock bass diets, it was also determined that those crayfish consumed 20 times more benthic biomass than smallmouth bass (Momot 1995). Thus orconectid crayfish may also compete with fish for benthic invertebrate resources, as found in a Utah reservoir (Hepworth and Duffield 1987) where *O. virilis* competes for food with the local trout population.

Despite the preponderance of crayfish in some fish diets, orconectid crayfish can also exert significant predation and competition effects on fish communities. *O. virilis* has been found to readily consume lake trout (*Salvelinus namaycush* [Walbaum]) sac fry and eggs (Horns and Magnuson 1981, Savino and Miller 1991). Further, experiments in Michigan have shown *O. virilis* preys heavily on bluegill eggs and larvae, reducing young-of-year recruitment (Dorn and Wojdak 2004, Dorn and Mittlebach 2004). Thus crayfish may inhibit recruitment in water bodies such as Lake Superior where lake trout densities, particularly egg densities, have dropped due to overfishing (Dorr et al. 1981). At low densities, further loss of eggs or fry may negatively impact future fish populations (Savino and Miller 1991).

Options for ecosystem management and mitigation of invasions

As orconectid crayfish re-establish in post-disturbance areas, and invade water bodies throughout Canada, it may be necessary to control or mitigate the effect they exert on primary and secondary productivity. Perhaps the most viable method of reducing crayfish populations will be top-down control using natural crayfish predators such as fish. Dorn and Mittelbach (1999) review the interactions between crayfish and fish extensively, and show that crayfish dominate the diets of many fish species. Predatory fishes will reduce crayfish abundance through direct predation (DiDonato and Lodge 1993, Garvey et al. 1994), non-consumptive increases in crayfish mortality (Hill and Lodge 1995), reduced foraging by surviving crayfish (Hill and Lodge 1994), and indirect competition with crayfish for zoobenthos. The two orconectid crayfish species reviewed here will likely be preyed upon strongly in Canada by smallmouth bass and rock bass (Rabeni 1992, Roell and Orth 1993, Garvey et al. 2003, Roth and Kitchell 2005). However, other species shown to opportunistically prey on *O. virilis* and *O. rusticus* are yellow perch (Roth and Kitchell 2005), walleye (Garvey et al. 2003, Roth and Kitchell 2005), and northern pike (Dorn and Mittelbach 1999) in addition to waterfowl and mammals - although this predation has not yet been quantitatively reported. Biocontrol practices could include managing fish harvests in water bodies on the fringe of *O. rusticus* or *O. virilis* invasions to allow retention of larger individual fish of these predatory species.

Roell and Orth (1993) have shown that adult fish prefer large mature crayfish

prey because they provide a greater foraging reward. As both *O. virilis* and *O. rusticus* are most reproductively active in their mature, adult life stages, control of crayfish populations may be most effective by populations of physically large crayfish-eating fish. However, many of Canada's water bodies have experienced intensive sport fishing, which has altered the community structure to favor small sizes that are less desirable to recreational fishermen. Thus many water bodies may lack fish populations capable of mitigating the negative impacts of crayfish introductions, and even face direct competition and predation from the orconectid crayfish. Smaller fish unable to consume adult crayfish due to gape size (Dorn and Mittelbach 1999) may become prey themselves, and will likely have the same prey base of zoobenthos as the crayfish.

THESIS OBJECTIVES

The primary objective of this thesis is to identify the potential impacts of reintroducing native crayfish species on the benthic environment of a post-acidification boreal lake. It is necessary to understand how re-introduction over the course of ecosystem recovery will alter the ecosystem that has developed since the removal of a former stressor. Such ecosystems may not be at the same ecological condition that formerly existed prior to acidification, and re-introducing a keystone species such as crayfish may cause dramatic ecological surprises.

Chapter two provides the examination of how *O. virilis* reintroduction may alter the benthic ecosystem of formerly acidified boreal lakes. I present the results of an enclosure/exclosure experiment that tested the hypothesis that the omnivorous

diet of *O. virilis* will lead to direct suppression of all trophic levels in a benthic food web (Fig. 1.3). I will further discuss the implications that re-introduction may have on the productive capacity of boreal lakes for sport fish harvesting.

Chapter three provides a summary of thesis findings, and more importantly a synthesis of what implications crayfish range expansion and re-introduction may have in Canada. I propose future directions for crayfish invasion research in Canada, in addition to areas where range expansion should be monitored. Finally, I discuss the management implications of species reintroduction to formerly perturbed ecosystems focusing on crayfish in watersheds from which they have been extirpated.

Table 1.1. Crayfish of Canada

Species	Provinces
Astacidae	
Pacifastacus leniusculus klamathensis (Stimpson)	BC
Pacifastacus leniusculus leniusculus (Dana)	BC
Pacifastacus leniusculus trowbridgii (Stimpson)	BC
Cambaridae	
Cambarus bartonii bartonii (Fabricius)	NB, ON, PQ
Cambarus diogenes Girard	ON
Cambarus robusticus Girard	ON
Fallicambarus fodiens (Cottle)	ON
Orconectes immunis (Hagen)	MB, ON, PQ
Orconectes limosus (Rafinesque)	ON
Orconectes obscurus (Hagen)	ON
Orconectes propinquus (Girard)	ON, PQ
Orconectes rusticus (Girard)	ON
Orconectes virilis Hagen	AB, SK, MB, ON, PQ, NB

Sources: Taylor et al. 1996, McAlpine et al. 1999

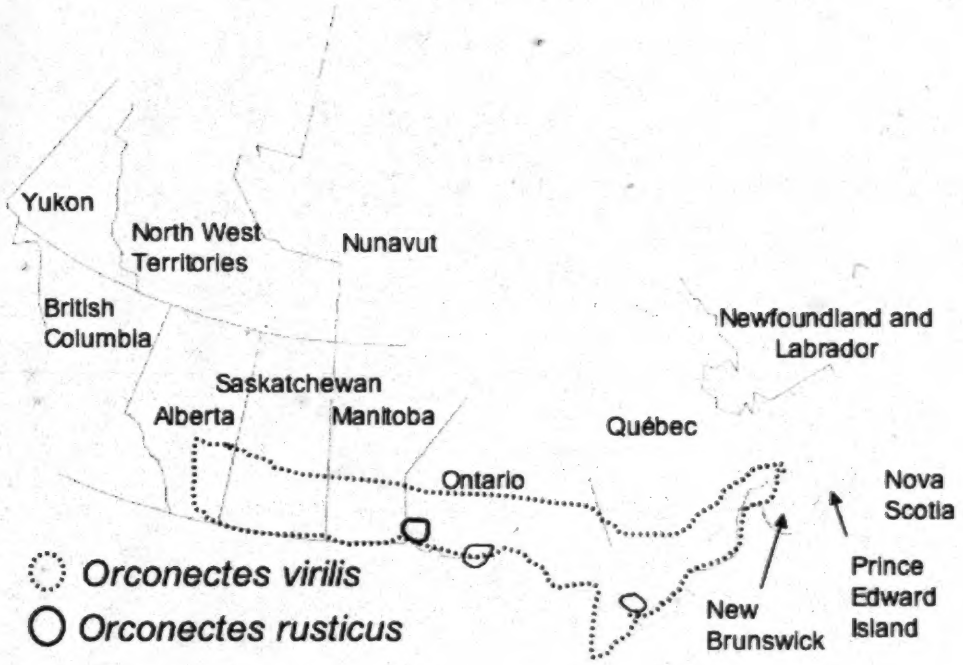

Figure 1.1. Approximate distribution of *Orconectes virilis* and *O. rusticus* in Canada. Adapted from Brock University Map Library.

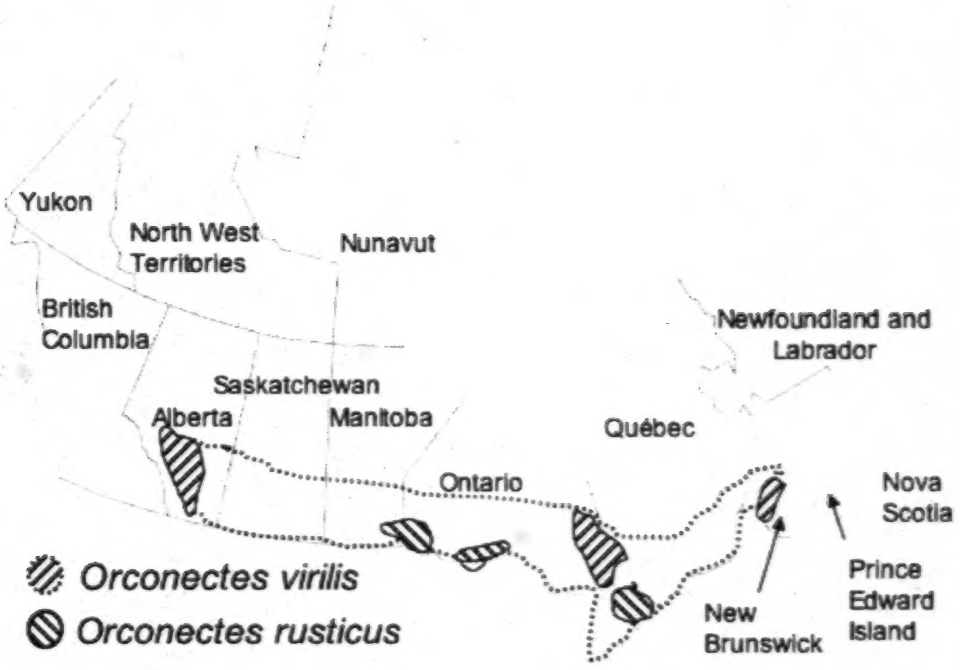

Figure 1.2. Areas of range expansion for *Orconectes virilis* and *O. rusticus* in Canada. Adapted from Brock University Map Library.

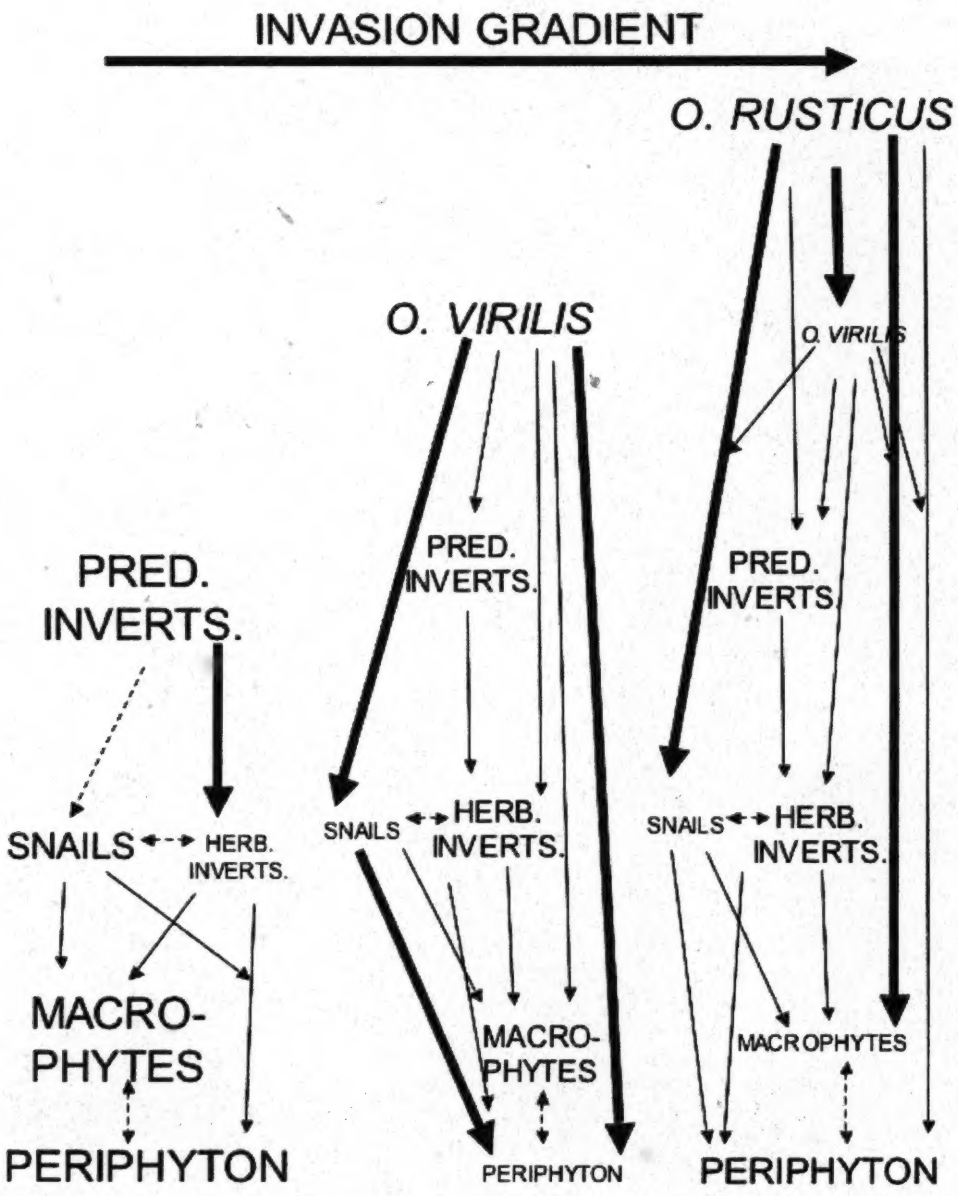

Figure 1.3. Hypothesized impacts of re-introduced (*Orconectes virilis*) and subsequent invasion by exotic (*Orconectes rusticus*) crayfish on littoral food webs in boreal lakes. — strong interaction. — moderate interaction. --- weak interaction, and --- in both directions indicates competition. PRED. INVERTS. = Predatory Invertebrates. HERB. INVERTS. = Herbivorous Invertebrates.

LITERATURE CITED

Abrahamsson, S. A. A. 1971. Density, growth and reproduction in populations of *Astacus astacus* and *Pacifastacus leniusculus* in an isolated pond. Oikos **22**:373-380.

Bergman, D. A., and P. A. Moore. 2003. Field observations of intraspecific agonistic behavior of two crayfish species, *Orconectes rusticus* and *Orconectes virilis*, in different habitats. Biological Bulletin **205**:26-35.

Berrill, M. 1978. Distribution and ecology of crayfish in the Kawartha lakes region of southern Ontario. Canadian Journal of Zoology **56**:166-177.

Brown, P. B., J. E. Wetzel, III, and A. Spacie. 1992. Evaluation of naturally occurring organisms as food for juvenile crayfish, *Procambarus clarkii*. Journal of the World Mariculture Society **23**:211-216.

Butler, M. J., and R. A. Stein. 1985. An analysis of the mechanisms governing species replacements in crayfish. Oecologia **66**:168-177.

Byron, C. J., and K. A. Wilson. 2001. Rusty crayfish (*Orconectes rusticus*) movement within and between habitats in Trout Lake, Vilas County, Wisconsin. Journal of the North American Benthological Society **20**:606-614.

Capelli, G. M., and B. L. Munjal. 1982. Aggressive interactions and resource competition in relation to species displacement among crayfish of the genus *Orconectes*. Journal of Crustacean Biology **2**:486-492.

Capelli, G. M., and J. J. Magnuson. 1983. Morphoedaphic and biogeographic analysis of crayfish distribution in northern Wisconsin. Journal of Crustacean Biology **3**:548-564.

Chambers, P. A., J. M. Hanson, J. Burke, and E. E. Prepas. 1990. The impact of the crayfish *Orconectes virilis* on aquatic macrophytes. Freshwater Biology **24**:81-91.

Charlebois, P. M., and G. A. Lamberti. 1996. Invading crayfish in a Michigan stream: direct and indirect effects on periphyton and macroinvertebrates. Journal of the North American Benthological Society **15**:551-563.

Clark, W. H., and G. T. Lester. 2005. Range extension and ecological information for *Orconectes virilis* (Hagen 1870) (Decapoda: Cambaridae) in Idaho, USA. Western North American Naturalist **65**:164-169.

Creaser, E. P. 1931. The Michigan decapod crustaceans. Papers of the Michigan Academy of Science, Arts, and Letters **13**:257-276.

Crocker, D. W., and D. W. Barr. 1968. Handbook of the crayfishes of Ontario. University of Toronto Press, Toronto, Ontario, Canada.

Crowl, T. A., and A. P. Covich. 1990. Predator-induced life-history shifts in a freshwater snail. Science **247**:949-951.

DiDonato, G. T., and D. M. Lodge. 1993. Species replacements among *Orconectes* crayfishes in northern Wisconsin lakes: the role of predation by fish. Canadian Journal of Fisheries and Aquatic Sciences **50**:1484-1488.

Dobson, A. P., J. P. Rodriguez, W. M. Roberts, and D. S. Wilcove. 1997. Geographic distribution of endangered species in the United States. Science **275**:550-553.

Dorn, N. J., and G. G. Mittelbach. 1999. More than predator and prey: a review of interactions between fish and crayfish. Vie et Milieu **49**:229-237.

Dorn, N. J., and G. G. Mittelbach. 2004. Effects of a native crayfish (*Orconectes virilis*) on the reproductive success and nesting behavior of sunfish (*Lepomis* spp.). Canadian Journal of Fisheries and Aquatic Sciences **61**:2135-2143.

Dorn, N. J., and J. M. Wojdak. 2004. The role of omnivorous crayfish in littoral communities. Oecologia **140**:150-159.

Dorr, J. A., III, D. V. O'Connor, N. R. Foster, and D. J. Jude. 1981. Substrate conditions and abundance of lake trout eggs in a traditional spawning area in southeastern Lake Michigan. North American Journal of Fisheries Management **1**:165-172.

Engel, S. 1990. Ecosystem responses to growth and control of submerged macrophytes: a literature review. Technical Bulletin Number 170, Wisconsin Department of Natural Resources, 20 pages.

Flecker, A. S. 1996. Ecosystem engineering by a dominant detritivore in a diverse tropical stream. Ecology **77**:1845-1854.

Garvey, J. E., and R. A. Stein. 1993. Evaluating how chela size influences the invasion potential of an introduced crayfish (*Orconectes rusticus*). American Midland Naturalist **129**:172-181.

Garvey, J. E., R. A. Stein, and H. M. Thomas. 1994. Assessing how fish predation and interspecific competition influence a crayfish assemblage. Ecology **75**:532-542.

Garvey, J. E., J. E. Rettig, R. A. Stein, D. M. Lodge, and S. P. Klosiewski. Scale-dependent associations among fish predation, littoral habitat, and distributions of

crayfish species. Ecology **84**: 3339-3348.

Gowing, H., and W. T. Momot. 1979. Impact of brook trout (*Salvelinus fontinalis*) predation on the crayfish *Orconectes virilis* in 3 Michigan lakes. Journal of the Fisheries Research Board of Canada **36**:1191-1196.

Hanson, J. M., P. A. Chambers, and E. E. Prepas. 1990. Selective foraging by the crayfish *Orconectes virilis* and its impact on the macroinvertebrates. Freshwater Biology **24**:481-491.

Heneberry, J. H., K. Errulat. M. Robataille, L. Whitmore, and J. R. M. Morris. 1992. A preliminary investigation on the distribution of freshwater crayfish (Decapoda: Cambaridae) in 32 lakes of the Sudbury Region of Northeastern Ontario. Project Report, Laurentian University, Sudbury, Ontario, Canada. 58 Pages.

Hepworth, D. K., and D. J. Duffield. 1987. Interactions between an exotic crayfish and stocked rainbow trout in Newcastle Reservoir, Utah. North American Journal Fisheries Management **7**:554-561.

Hill, A. M., and D. M. Lodge. 1994. Diel changes in resource demand: competition and predation in species replacement among crayfishes. Ecology **75**:2118-2126.

Hill, A. M., and D. M. Lodge. 1995. Multi-trophic-level impact of sublethal interactions between bass and omnivorous crayfish. Journal of the North American Benthological Society **14**:306-314.

Hill, A. M., and D. M. Lodge. 1999. Replacement of resident crayfishes by an exotic crayfish: the roles of competition and predation. Ecological Applications **9**:678-690.

Hill, A. M., D. M. Sinars, and D. M. Lodge. 1993. Invasion of an occupied niche by the crayfish *Orconectes rusticus*: potential importance of growth and mortality. Oecologia **94**:303-306.

Hobbs III, H. H, J. P. Jass, and J. V. Hunter. 1989. A review of global crayfish introductions with particular emphasis on two North American species (Decapoda, Cambaridae). Crustaceana **56**:299-316.

Horns, W. H., and J. J. Magnuson. 1981. Crayfish predation on lake trout eggs in Trout Lake, Wisconsin. Rapports et Proces-Verbaux des Reunions Conseil International pour l'Exploration de la Mer **178**:299-303.

Huryn, A. D., and J. B. Wallace. 1987. Production and litter processing by crayfish in an Appalachian mountain stream. Freshwater Biology **18**:277-286.

Jones, P. D., and W. T. Momot. 1983. The bioenergetics of crayfish in two pothole lakes. Freshwater Crayfish **5**:193-209.

Keller, W., J. H. Heneberry, and J. M. Gunn. 1999. Effects of emission reductions from the Sudbury smelters on the recovery of acid- and metal-damaged lakes. Journal of Aquatic Ecosystem Stress and Recovery **6**:189-198.

Kershner, M. W., and D. M. Lodge. 1995. Effects of littoral habitat and fish predation on the distribution of an exotic crayfish, *Orconectes rusticus*. Journal of the North American Benthological Society **14**:414-422.

Kondolf, G. M. 2000. Assessing salmonid spawning gravel quality. Transactions of the American Fisheries Society **129**: 262-281.

Lawton, J. H. 1994. What do species do in ecosystems? Oikos **71**:367-374.

Lodge, D. M. 1993*a*. Species invasions and deletions: community effects and responses to climate and habitat change. In: Karieva, P., Kingsolver, J., and Huey, R. (eds). Biotic Interactions and Global Change. Sinauer, Sunderland, Massachusetts, pp 367-387.

————. 1993*b*. Biological invasions: lessons for ecology. Trends in Ecology and Evolution **8**:133-137.

Lodge, D. M. and J. G. Lorman. 1987. Reductions in submersed macrophyte biomass and species richness by the crayfish *Orconectes rusticus*. Canadian Journal of Fisheries and Aquatic Sciences **44**:591-597.

Lodge, D. M., T. K. Kratzand, G. M. Capelli. 1986. Long term dynamics of three crayfish species in Trout Lake, Wisconsin. Canadian Journal of Fisheries and Aquatic Sciences **43**:993-998.

Lodge, D. M., M. W. Kershner, J. E. Aloi, and A. P. Covich. 1994. Effects of an omnivorous crayfish (*Orconectes rusticus*) on a freshwater littoral food web. Ecology **75**:1265-1281.

Lodge, D. M., C. A. Taylor, D. M. Holdich, and J. Skrudal. 2000. Nonindigenous crayfishes threaten North American freshwater biodiversity: lessons from Europe. Fisheries **25**:7-19.

Lorman, J. G. 1980. Ecology of the crayfish *Orconectes rusticus* in northern Wisconsin. PhD. Thesis, University of Wisconsin, Madison, WI. 227 pages.

Mather, M. E., and R. A. Stein. 1993. Direct and indirect effects of fish predation on the replacement of a native crayfish by an invading congener. Canadian Journal of Fisheries and Aquatic Sciences **50**:1279-1288.

McAlpine, D. F., T. J. Fletcher, M. A. Osepchook, and J. C. Savoie. 1999. A range extension for *Orconectes virilis* (Decapoda, Cambaridae) and a third crayfish species for New Brunswick, Canada. Crustaceana **72**:356-358.

McCarthy, J. M., C. L. Hein, J. D. Olden, and M. J. Vander Zanden. 2006. Coupling long-term studies with meta-analysis to investigate impacts of non-native crayfish on zoobenthos communities. Freshwater Biology **51**:224-235.

McNaught, A. S., D. W. Schindler, B. R. Parker, A. J. Paul, R. S. Anderson, D. B. Donald, and M. Agbeti. 1999. Restoration of the food web of an alpine lake following fish stocking. Limnology and Oceanography **44**:127-136.

Miller, R. R., J. D. Williamsand, J. E. Williams. 1989. Extinctions of North American fishes during the last century. Fisheries **14**:22-38.

Mittlebach, G. G. 1981. Foraging efficiency and body size: a study of optimal diet and habitat use by bluegills. Ecology **62**:1370-1386.

Momot, W. T. 1984. Crayfish production: a reflection of community energetics. Journal of Crustacean Biology **4**:35-54.

Momot, W. T. 1992. Further range extensions of the crayfish *Orconectes rusticus*

in the Lake Superior Basin of northwestern Ontario. The Canadian Field Naturalist **106**:397-399.

Momot, W. T. 1995. Redefining the role of crayfish in aquatic ecosystems. Reviews in Fisheries Science **3**:33-63.

Momot, W. T., and H. Gowing. 1977. Results of an experimental fishery on the crayfish *Orconectes virilis*. Journal of the Fisheries Research Board of Canada **34**:2056-2066.

Momot, W. T., H. Gowing, and P. D. Jones. 1978. The dynamics of crayfish and their role in aquatic ecosystems. The American Midland Naturalist **99**:10-35.

Momot, W. T., C. Hartviksen, and G. Morgan. 1988. A range extension for the crayfish *Orconectes rusticus*: Sibley Provincial Park, northwestern Ontario (Canada). The Canadian Field Naturalist **102**:547-548.

Nelson, J. S., and M. J. Paetz. 1992. The Fishes of Alberta. 2nd Edition. The University of Alberta Press, Edmonton, Alta.

Nyström, P., C. Brönmark, and W. Granéli. 1999. Influence of an exotic and a native crayfish species on a littoral benthic community. Oikos **85**:545-553.

Olsen, T. M., D. M. Lodge, G. M. Capelli, and R. J. Houlihan. 1991. Mechanisms of impact of an introduced crayfish (*Orconectes rusticus*) on littoral congeners, snails, and macrophytes. Canadian Journal of Fisheries and Aquatic Sciences **48**:1836-1846.

Page, L. M. 1985. The crayfishes and shrimps (Decapoda) of Illinois. Illinois Natural History Survey Bulletin **33**:335-448.

Parkyn, S. M., C. F., Rabeni, and K. J. Collier. 1997. Effects of crayfish (*Paranephrops planifrons*: Parastacidae) on in-stream processes and benthic faunas: a density manipulation experiment. New Zealand Journal of Marine and Freshwater Research **31**:685-692.

Perry, W. L., D. M. Lodge, and G. A. Lamberti. 1997. Impact of crayfish predation on exotic zebra mussels and native invertebrates in a lake-outlet stream. Canadian Journal of Fisheries and Aquatic Sciences **54**:120-125.

————. 2000. Crayfish (*Orconectes rusticus*) impacts on zebra mussel (*Dreissena polymorpha*) recruitment, other macroinvertebrates and algal biomass in a lake-outlet stream. American Midland Naturalist **144**:308-316.

Perry, W. L., D. M. Lodge, and J. L. Feder. 2001. Hybrid zone dynamics and species replacement between *Orconectes* crayfishes in a northern Wisconsin lake.

Evolution **55**:1153-1166.

Perry, W. L., J. L. Feder, and D. M. Lodge. 2002. Importance of hybridization between indigenous and nonindigenous freshwater species: and overlooked threat to North American biodiversity. Systematic Biology **51**:255-275.

Probst, W. E., C. F. Rabeni, W. G. Covington, and R. E. Marteney. 1984. Resource use by stream-dwelling rock bass and smallmouth bass. Transactions of the American Fisheries Society **113**:283-294.

Puth, L. M., and T. F. H. Allen. 2004. Potential corridors for the rusty crayfish, *Orconectes rusticus*, in northern Wisconsin (USA) Lakes: lessons for exotic invasions. Landscape Ecology **20**:567-577.

Rabeni, C. F. 1992. Trophic linkage between stream centrarchids and their crayfish prey. Canadian Journal of Fisheries and Aquatic Sciences **49**:1714-1729.

Roell, M. J., and D. J. Orth. 1993. Trophic basis of production of stream-dwelling smallmouth bass, rock bass, and flathead catfish in relation to invertebrate bait harvest. Transactions of the American Fisheries Society **122**:46-62.

Roth, B. M., and J. F. Kitchell. 2005. The role of size-selective predation in the displacement of *Orconectes* crayfishes following rusty crayfish invasion.

Crustaceana **78**:297-310.

Saffran , K. A., D. R. Barton. 1993. Trophic ecology of the crayfish *Orconectes propinquus* in Georgian Bay. Freshwater Crayfish **9**:350-358.

Savino, J. F., and J. E. Miller. 1991. Crayfish (*Orconectes virilis*) feeding on young lake trout (*Salvelinus namaycush*): effect of rock size. Journal of Freshwater Ecology **6**:161-170.

Statzner, B., U. Fuchs, and L. W. G. Higler. 1996. Sand erosion by mobile predaceous stream insects: implications for ecology and hydrology. Water Resources Research **32**:2279-2287.

Statzner, B., M.- F. Arens, J.- Y. Champagne, R. Morel, and E. Herouin. 1999. Silk-producing stream insects and gravel erosion: significant biological effects on critical shear stress. Water Resources Research **35**:3495-3506.

Statzner, B., E. Fièvet, J. Champagne, R. Moreland, E. Herouin. 2000. Crayfish as geomorphic agents and ecosystem engineers: Biological behavior affects sand and gravel erosion in experimental streams. Limnology and Oceanography **45**:1030-1040.

Statzner, B., O. Peltret, and S. Tomanova. 2003. Crayfish as geomorphic agents and

ecosystem engineers: effect of a biomass gradient on baseflow and flood-induced transport of gravel and sand in experimental streams. Freshwater Biology **48**:147-163.

Stein, R. A. 1977. Selective predation, optimal foraging, and the predator-prey interactions between fish and crayfish. Ecology **58**:1237-1253.

Stewart, T. W., J. G. Miner, and R. L. Lowe. 1998. An experimental analysis of crayfish (*Orconectes rusticus*) effects on a *Dressena*-dominated benthic macroinvertebrate community in western Lake Erie. Canadian Journal of Fisheries and Aquatic Sciences **55**:1043-1050.

Sullivan, M. G. 2003. Active management of walley fisheries in Alberta: dilemmas of managing recovering fisheries. North American Journal of Fisheries Management **23**:1343-1358.

Taugbøl, T., and S. Peay. 2004. Roundtable session 3: Reintroduction of native crayfish and habitat restoration. Bulletin Français de la Pêche et de la Pisciculture **372-373**:465-471.

Taylor, C. A., M. Redmer. 1996. Dispersal of the crayfish *Orconectes rusticus* in Illinois, with notes on species displacement and habitat preference. Journal of Crustacean Biology **16**:547-551.

Taylor, C. A., M. L. Warren, Jr., J. F. Fitzpatrick, Jr., H. H. Hobbs, III, R. F. Jezerinac, W. L. Pflieger, and H. W. Robinson. 1996. Conservation status of crayfishes of the United States and Canada. Fisheries **21**:25-38.

Telford, M. 1970. Comparative carbohydrase activities of some crustacean tissue and whole animal homogenates. Comparative Biochemistry and Physiology **34**:81-90.

Vitousek, P. M., C. M. D'Antonio, L. L. Loope, and R. Westbrooks. 1996. Biological invasions as global environmental change. American Scientist **84**:468-477.

Webster, J. R. and B. C. Patten. 1979. Effects of watershed perturbation on stream potassium and calcium dynamics. Ecological Monographs **49**:51-72.

Williamson, M., and A. Fitter. 1996. The varying success of invaders. Ecology **77**:1661-1666.

Wilson, K. A. 2002. Impacts of rusty crayfish (*Orconectes rusticus*) in northern Wisconsin lakes: 1-194 (Ph.D. Thesis, University of Wisoconsin-Madison).

Wilson, K. A., J. J. Magnuson, D. M. Lodge, A. M. Hill, T. K. Kratz, W. L. Perry,

and T. V. Willis. 2004. A long-term rusty crayfish (*Orconectes rusticus*) invasion: dispersal patterns and community change in a north temperate lake. Canadian Journal of Fisheries and Aquatic Sciences **61**:2255-2266.

Wotton, R. S., B. Malmqvst, T. Muotka, and K. Larsson. 1998. Fecal pellets from a dense aggregation of suspension-feeders in a stream: an example of ecosystem engineering. Limnology and Oceanography **43**:719-725.

CHAPTER TWO

RE-INTRODUCTION OF CRAYFISH TO A RECOVERING ACIDIFIED LAKE: ECOLOGICAL RESTORATION OR INVASION?

INTRODUCTION

As ecosystems recover from environmental perturbations, trophic interactions are expected to replace abiotic factors as key ecological drivers (Menge and Sutherland 1987, Hogsden and Vinebrooke 2005). In particular, re-establishment of functionally important species is considered a key event in the recovery of perturbed ecosystems, potentially reversing catastrophic state shifts (Scheffer et al. 2001, Power et al. 1996). A common assumption is that functionally important species will re-colonize naturally once environmental conditions are amenable to their existence (Bohonak and Jenkins 2003). For example, many benthic arthropods are able to rapidly re-colonize recovering acidified lakes owing to their aerial dispersal capabilities as adults (Keller et al. 1992, Carbone et al. 1998, Bohonak and Jenkins 2003). However, if dispersal barriers exist, then extirpated resident species will remain absent despite environmental improvements.

Re-introduction of functionally important species is a potential strategy for reversing catastrophic ecosystem shifts. For example, re-introduction of a dominant zooplankter (*Hesperodiaptomus arcticus* Marsh) extirpated by past stocking of sportfish was necessary to restore an alpine lake to its natural state (McNaught et al. 1999). Other re-introduction programs have been implemented to restore extirpated populations such as black bear (*Ursus americanus*) (Smith and Clark 1994) and

wolves in Yellowstone National Park, U.S.A. (Varley and Boyce 2006). However, little research has been done on the effects that returning species will have on the ecosystem that has developed since their extirpation. Nor has much attention been committed to the possibility of animal reintroduction as a restoration strategy in ecosystem management outside of proposed crayfish re-introduction strategies in Europe (Taugbøl and Peay 2004).

Crayfish are omnivores that play key roles in many benthic food webs in lakes and streams (Souty-Grosset 2005), consuming invertebrates, macrophytes, detritus, and periphyton (Olsen et al. 1991, Lodge et al. 1994, Charlebois and Lamberti 1996, Nyström et al. 1996, 1999). However, crayfish can also show selective feeding depending on prey availability and its vulnerability (Nyström et al. 1999). Unlike obligate top predators that exert strong top-down forces on benthic food chains (Power 1990, Brönmark et al. 1992, Brönmark 1994), omnivores have more diffuse effects on lower trophic levels, which can confound predictions of trophic cascades (Polis and Holt 1992, Power 1992, Vinebrooke et al. 2001). The food-web impacts of several crayfish species, such as *Orconectes virilis* (Hagen) (Dorn and Wojdak 2004), *Orconectes rusticus* (Girard) (Lodge et al. 1994), and *Pacifastacus leniusculus* (Dana) (Nyström et al. 1996), have been shown to deviate from the classic trophic cascade model. Therefore, it is difficult to predict the exact impact that an omnivorous crayfish would have once it is re-introduced into a littoral food web.

The crayfish *Orconectes virilis* is a large omnivorous invertebrate sensitive to acidification (Keller et al. 1999). In experimental whole lake manipulations

(Davies 1989) whole populations of *O. virilis* collapsed when pH declined below ~6.0. Boreal regions of Northeastern Ontario that experienced landscape-scale acid deposition and associated lowered lake pH also showed declines in crayfish abundance (Heneberry et al. 1992, France 1993, Keller et al. 1999). Despite the dramatic reduction in SO_2 emissions since the 1970's, and associated chemical and biological improvements (Gunn and Keller 1990, Keller et al. 1999), *O. virilis* has not re-colonized many lakes it likely occupied prior to acidification, and the absence of key species such as this are a concern for the potential recovery of these previously severely stressed systems (Keller et al. 1999).

The possibility of using bio-manipulation to return ecosystems to their natural state requires knowledge of how such reintroductions would change the benthic community from its novel condition to the community that existed prior to the perturbation. The main goal of my experiment was to determine the impact of re-introduced crayfish on the littoral food web of a recovering acidified lake. I hypothesized that if a natural population density of *O. virilis* was re-introduced into a lake, it would suppress large predacious odonates and periphyton, both of which proliferate following the extirpation of higher trophic levels (e.g., fish) during acidification (Schindler et al. 1991) and subsequently persist during the advanced stages of recovery (Fig. 2.1). If crayfish exert striking impacts, such as elimination of species and suppression of periphyton, then I consider whether re-introduction of this species would constitute a restoration effort or an invasion. The most dramatic invaders not only consume native species and compete for resources, but also modify physical habitat (Jones et al. 1994) and trophic interactions.

METHODS

Study site

The experiment was conducted along the southwest shore of a headwater lake named Lake 302 South (L302S) (lat 93°45′W, long 49°40′N), Experimental Lakes Area (ELA), Ontario, Canada (see Vinebrooke et al. 2001 for a full site description). L302S underwent rigorous pH manipulation using H_2SO_4 as part of an acidification experiment (Schindler et al. 1991, Turner et al. 1995*a*, 1995*b*) wherein the southern basin was curtained off, and its pH dropped from 6.8 to 4.5 during 1982 to 1991. The native crayfish species *Orconectes virilis* was extirpated from L302S once pH dropped below 5.1 (Vinebrooke et al. 2001). The lake pH has returned to pre-acidification levels, and much of the fish assemblage has been restored (see Vinebrooke et al. 2001). The curtains are still in place, and together with an outflow impassable to crayfish, have prevented the re-colonization of L302S by *O. virilis* from downstream Lake 468 (L468).

Experimental design

A single-factor experimental design consisting of two treatment levels (crayfish-less control vs. 2 crayfish/m^2) was replicated five times for a total of 10 aquaculture enclosures. Each cage had a floor benthic area of $4m^2$ and was located along a 1-m depth contour where a predominantly sandy substrate was covered by a thin organic layer. The experiment ran for 56 days from 17 June to 12 August 2004.

Cobble baskets (n = 12) were introduced into each enclosure to simulate the

natural cobble substrate environment of the lake, and provide discrete sampling units for invertebrates. Each open basket (0.25 m x 0.25 m x 0.15 m) was constructed using 5-cm mesh-sized steel hardware cloth. The floor of each basket was reinforced with two pine dowels crossing from corner to corner secured with plastic straps. Eighty-pound test fishing line was used as a handle for the basket strung from the corners and buoyed the handle in the water column with a small styrofoam float. Each basket was filled with cobble collected from L302S, and four unglazed ceramic tiles (upper surface area = 53.29 cm^2) provided a standard substrate for sampling of periphyton. Cobble baskets and tiles were pre-conditioned in the littoral zone for a 3-week period prior to the start of the crayfish re-introductions.

Crayfish introductions

Crayfish were collected from L468 using night littoral surveys, and SCUBA dives from 13 - 16 June, 2004. As it is the lake immediately downstream of L302, L468 *O. virilis* populations were likely the most closely related group of crayfish to those in L302 prior to extirpation, and the most probable source population for re-colonization of L302S in the future. Adult male crayfish (mean carapace length: 14.02 mm ± 1.39 SD) were introduced into the enclosures on 17 June at a concentration of ~ 1.8 individuals/m^2. Natural densities of *O. virilis* in other lakes have been found to vary from 1.5 – 2.6 individuals/m^2 (Momot and Gowing 1977) to 0.2-0.6 individuals/m^2 (Davies 1989, France and Collins 1993). Similar crayfish introduction experiments conducted by Dorn and Mittelbach (2004) and Dorn and Wojdak (2004) employed *O. virilis* densities of 1.4 and 1.5 individuals/m^2.

Zoobenthos sampling

Three cobble baskets were randomly selected from each enclosure, and sampled at 2-wk intervals for periphyton and zoobenthos. Tiles were placed in a sealed container while at the bottom of the enclosure to prevent physical disturbance as the basket itself was removed. Cobble baskets were gently scooped into a plastic container and lifted through the water column to the surface. As the basket was in an enclosed container when removed I am confident invertebrate losses due to basket removal were minimal, or at least consistent across both treatments.

Cobble was scrubbed by hand to dislodge invertebrates, and the resulting mixture was concentrated through a 90-µm sieve. Samples were immediately placed on ice and sorted back at the laboratory. Invertebrates were identified to the lowest possible taxon under a dissecting microscope using keys (Merritt and Cummins 1996, Wiggins 1996, Clifford 1991, Davies 1971), and their body length was measured to the nearest 1 mm. Intact invertebrates were freeze-dried and weighed to determine dry weights, while the weights of partially damaged individuals were calculated from the body length – dry weight conversion equations of Benke et al. (1999) and expressed as the sum of the three cobble baskets for each enclosure. Most invertebrates were in adequate condition for individual weighing, and equations were used rarely.

Periphyton

All four tiles from each sampled cobble basket were pooled and periphyton

was harvested on each sampling date using a toothbrush, and then filtered onto a Whatman GF/C filter (pore size = 1.2 µm). Algal biomass was quantified using ash-free dry weight (AFDW) as a proxy. The filters were freeze-dried, periphyton scraped off and homogenized with a mortar and pestle. AFDW was then determined for each sampling day by combusting a sub-sample and determining differences between dry and ash (at 500°C for 24 h) weights. Periphyton pigment concentrations were quantified using a reversed-phase, high-pressure, liquid chromatography (HPLC) procedure (Vinebrooke and Leavitt 1999). Pigments were extracted by soaking freeze-dried periphyton sub-samples in acetone, methanol, and water (80:15:5 v/v) for 24 h in darkness at -10°C. Extracts were filtered through 0.2-µm pore nylon filters, dried, and stored under nitrogen gas in the dark at -10°C. Dried extracts were reconstituted using injection solution (70% acetone: 25% ion-pairing reagent: 5% methanol). Pigments were separated on a Agilent (Hewlett-Packard Canada Ltd., Mississauga, Ont.) Model 1100 system with a Varian Microsorb™ Model 100 column (10-cm length, 3-µm particle size). Pigments were detected with an inline HP Series 1100 diode array detector (435-nm detection wavelength) and a fluorescence detector (435-nm excitation wavelength, 667-nm detection wavelength).

Leaf detritus

Leaf detritus was included in the form of leaf packs to identify whether detritus was a preferred food source for *O. virilis*. Fallen leaves were collected from the riparian zone around L302S prior to the launch of the experiment. Birch and

alder leaves were sorted out from the leaf litter and air-dried to a constant weight. Leaves were enclosed in loose mesh bags (2.51 cm opening) and weighed to provide leaf packs of 23.1±3.9g. Five leaf packs were included in each enclosure, and attached to cobble baskets with plastic cable ties. All leaf packs were sampled at day 56, at the conclusion of the experiment. Once the leaf packs wee removed they were and oven dried at 50°C and reweighed until they dried to a constant weight.

Gut-content analysis

All crayfish recovered from the enclosures were dissected and their foreguts removed. Only contents of the foregut were examined as nearly all material in the mid- and hind- guts were digested beyond recognition. The foregut of each crayfish was excised and its contents emptied into a water-filled, 1 mm grid lined petri dish, stirred to distribute evenly, and viewed with a dissecting microscope. Gut contents were classified as particulate detritus, algae, or invertebrate. Percent total area of the dish covered by each food-type was estimated using a 1-mm^2 grid. Sub-samples were taken and examined with a compound microscope to confirm the identity of items. All invertebrate individuals were identified to the most specific extent possible. Immediately subsequent to dissection, the remaining crayfish material was refrozen for stable isotope analysis. At the end of the experiment, 23 adult male crayfish were collected from the source lake (L468), and analyzed for gut-contents and stable isotopes to identify how close the diets of experiment crayfish were to a local population of *O. virilis*.

Stable isotope analyses

Stable isotope analysis is an alternative method to identify the primary carbon source, and elucidate the trophic structure in a food web (Peterson and Fry 1987, Whitledge and Rabeni 1997). Stable isotope ratios in an organism reflect all food sources consumed over the period of time required for elemental turn over in that organism's tissues (Kling et al. 1992). Because the ratio of $\delta^{13}C$ to $\delta^{12}C$ changes little with increasing trophic level it is useful to track the flow of energy through a benthic food web, and can determine the importance of ultimate C sources to primary consumers and upper-level trophic organisms (Hecky and Hesslein 1995).

Stable nitrogen ratios, however, are altered between each consecutive consumer level. The $\delta^{15}N/\delta^{14}N$ ratio of an organism also reflects the $\delta^{15}N/\delta^{14}N$ of its food sources, although consumers assimilate $\delta^{15}N$ preferentially over $\delta^{14}N$ in their diet (DeNiro and Epstein 1978). The heavier isotope, $\delta^{15}N$, is enriched 3 to 5‰ as N is passed from prey to predator, and $\delta^{15}N$ enrichment is consistent with increasing trophic level (Minagawa and Wada 1984). Therefore, one can potentially identify relative trophic position of the organisms present in an environment. Integrating the two stable isotope elements allows identification of the original carbon source for a community, and evaluation of the trophic relationship among organisms (Hobson and Welch 1992).

Both stable isotopes and gut-content analysis can provide reliable quantitative estimates of the contributions of energy sources to production of crayfish and other aquatic organisms. Whitledge and Rabeni (1997) found crayfish

dietary trophic position calculated from gut content analysis correlates well with their trophic position determined from tissue $\delta^{15}N$. However, whereas gut analysis only provides a snapshot of what an organism consumed immediately prior to capture, stable isotope analyses integrate a long timeframe of dietary consumption.

In order to obtain the biomass required for the analyses, taxa were pooled into their respective families, and across the ten replicates for each sampling date. Chironomids were separated into tanypodine and non-tanypodine chironomids as Tanypodinae is the only taxa in Chironomidae that is predominantly carnivorous (Merritt and Cummings 1996), and their trophic function may have differed from other chironomids in the enclosures. Although the variance of isotopic composition across replicates can no longer be determined when one pools the organisms, the average isotopic composition of taxa at a given time can still be shown (Rosenfeld and Roff 1992, Nyström et al. 1999). All periphyton and zoobenthos samples were frozen immediately after they were sorted. Stable isotope analysis was also conducted on abdominal muscle tissue of all crayfish recovered from the enclosures. All samples were homogenized by grinding into a fine powder with a mortar and pestle. Isotopic analysis was performed at the Biogeochemical Analysis Laboratory, University of Alberta.

Statistical analyses

Repeated-measures (RM) ANOVAs and RM-MANOVAs were conducted to test for the time-dependent effects of crayfish re-introduction on total community biomass and taxonomic composition, respectively. Significant RM-ANOVA results

were further examined using individual RM-ANOVAs to identify the effects of crayfish on individual zoobenthos taxa, and taxonomically diagnostic algal pigments. Analyses were performed using SPSS software (version 11.0.3, SPSS Inc., Chicago, Illinois).

RESULTS

Effects of O. virilis on zoobenthos

Crayfish significantly suppressed total invertebrate biomass (RM-ANOVA, treatment effect, $p < 0.05$) (Table 2.1.a, 2.1.b, 2.2, Fig. 2.2) and altered its taxonomic composition (RM-MANOVA, treatment effect, $p = 0.003$). RM-ANOVAs revealed that the negative impact of crayfish on zoobenthos abundance was attributable to its effects on odonates, leeches, and chironomids (Table 2.2, Fig. 2.3). In contrast, crayfish did not significantly affect the abundance of ephemeropterans or trichopterans (Fig. 2.3).

Crayfish exerted contrasting taxon-specific effects on odonates (Table 2.2, Fig. 2.3). *O. virilis* significantly suppressed the total biomass of the most abundant odonate, namely *Aeshna*. However, crayfish significantly stimulated the population density of this anisopteran (RM – ANOVA, $F = 4.37$, $p = 0.04$; Fig. 2.4). In contrast, the presence of crayfish significantly increased the total zygopteran biomass, consisting primarily of *Enallagma* (Table 2.2, Fig. 2.3).

Effects of O. virilis on periphyton and leaf litter

The presence of crayfish significantly decreased AFDW-inferred total

periphyton biomass (Table 2.2, Fig. 2.5), and altered the taxonomic composition of the algal community (RM-MANOVA, treatment-time interaction, Pillai's Trace p = 0.03). There is no significant difference of the (RM-ANOVA p>0.05 Treatment) contribution of Chl *a* to dry mass of periphyton between the enclosures and exclosures (Fig. 2.6) indicating that both firmly attached algal and loosely attached detrital components of the periphyton matrix were affected equally by the presence of crayfish. Further, RM-ANOVAs revealed that *O. virilis* suppressed chlorophyll-inferred algal biomass and altered its taxonomic composition by disproportionately reducing chlorophyll *b* and lutein, which are taxonomically diagnostic pigments associated with the filamentous green algae (Table 2.3, Fig. 2.7). Similarly, crayfish significantly suppressed fucoxanthin and diatoxanthin concentrations, which indicated a reduced abundance of benthic diatoms. In contrast, there was no significant effect of crayfish on dry biomass of the leaf packs by the end of the experiment (Student's T-test, p >0.05).

Gut and stable isotopic analyses

As live measurements were made at the termination of the experiment, too much time lapsed and what material was present in their guts had been digested. However, two crayfish from one enclosure had large amounts of crayfish carapace in their fore gut which likely originated from a freshly molted individual in this group.

Introduced crayfish in L302S and the source population from L468 occupied intermediate trophic positions between primary and secondary consumers based on their $\delta^{15}N$ signatures and a standard enrichment of 3.4‰ per trophic level (Fig. 2.8).

In comparison, predatory leeches (*N. obscura*) had slightly enriched $\delta^{15}N$ values, while these were similar among the odonates *Aeshna*, and *Enallagma* and the crayfish. Other invertebrates that also occupied the same intermediate trophic position were the tanypodine chironomids and trichopterans. Primary consumers included herbivorous ephemeropterans and non-tanypodine chironomids.

Periphyton was the primary carbon source to most benthic invertebrates based on their well-aligned $\delta^{13}C$ values (Fig. 2.8). In contrast, leaf detritus did not constitute a major dietary component of the benthos. *O. virilis* from the enclosures and Lake 468 were enriched in $\delta^{13}C$ by ~ 3‰ relative to the rest of the food web.

DISCUSSION

Many of the food-web impacts that I hypothesized for crayfish in recovering acidified lakes (Fig. 2.1) were supported by evidence from the experimental re-introduction of naturally low densities of *O. virilis*. In particular, crayfish reduced total zoobenthos biomass by 70% through suppression of large predatory odonates. In contrast, smaller predatory invertebrates benefited from the presence of crayfish, while the biomass of herbivorous invertebrates were unaffected. Nevertheless, periphyton biomass was reduced by 90%, suggesting a direct effect of crayfish. Below, I consider both possible ecological mechanisms underlying the observed food-web impacts of crayfish, and their potential ramifications for restoration of the damaged lake ecosystems.

Impacts of O. virilis on zoobenthos

Our findings support other reports that crayfish function as strong benthivores in freshwater lakes (Hanson et al. 1990, Lodge et al. 1994, McCarthy et al. 2006) and streams (Helms and Creed 2005). In particular, *O. virilis* strongly suppressed the total abundance of the anisopteran *Aeshna* by negatively affecting larger individuals belonging to this genus. However, densities of *Aeshna* were significantly elevated in the enclosures relative to the crayfish-less controls. Similarly, the smaller zygopteran genus *Enallagma* was also more abundant in the presence of crayfish. Therefore, I suggest that size-selective predation by crayfish on larger odonates, or possibly their active avoidance of this large predator, released smaller odonates from competition and cannibalism (Stoks and McPeek 2003*a*, *b*).

As Stenroth et al. (2006) have suggested that tissue turnover rates in crayfish may be slow enough to cover differences in diet between months, it is possible that crayfish predation in the experiment may have been masked by the diet of the crayfish in their source lake. Despite this potential limitation, I can certainly reconstruct the rest of the food web present in the enclosures with stable isotopes (Fig. 2.8), and when used in combination with the quantified biomass effects of the crayfish develop a reliable picture of crayfish reintroduction effects on benthic communities. Evidence from stable isotopic analysis and the lack of odonate remains in the guts of source lake crayfish (ID Phillips personal observation) suggest that active avoidance rather than predation better explains why crayfish suppressed large odonates in the enclosures. *O. virilis* did not exhibit an elevated $\delta^{15}N$ signal relative to odonates (Fig. 2.8), suggesting that odonates did not constitute a major

component of the crayfish diet.

Johnson (1991) described the necessity of refugia for odonates in their efforts to find a balance between actively foraging, and hiding from predators. It is possible that crayfish excluded large anisopterans from decent refugia, thereby increasing their susceptibility to predation by resident minnows (Vinebrooke et al. 2001). Further, Kirk and Smock (2000) observed that competition for physical refugia was intense between crayfish and large predacious invertebrates, while crayfish often shared these spaces with smaller individuals of the same invertebrate. Therefore, crayfish likely excluded large odonates from the enclosures owing to a combination of predation and competition. Further, competitive interference could also account for why crayfish significantly suppressed leeches, which occupied a similar trophic position as *O. virilis* based stable isotopic evidence (Fig. 2.8).

A potential explanation for the observed concomitant decreases in large instars of the odonate *Aeshna*, and increases in younger instars of *Aeshna* and smaller *Enallagma* is release from predation and competition owing to size-selective displacement by crayfish. Large odonate larvae have the potential to out-compete small larvae for prey, or quite simply prey on smaller larvae (Benke et al. 1982, Morin 1984). Interestingly, the closely related invasive rusty crayfish *O. rusticus* exerted the opposite negative effect on *Enallagma* (McCarthy et al. 2006) by eliminating its preferred macrophyte habitat (Dionne and Folt 1991, Hilsenhoff 1995). These species-specific effects of *Orconectes* highlight how native and exotic congeners can exert contrasting impacts in freshwater ecosystems.

Our hypothesized direct link between *O. virilis* and the herbivores (Fig. 2.1)

was not realized, but *O. virilis* certainly had such a dramatic effect as a herbivore itself (Fig. 2.5) that a trophic impact on the primary consumer taxa was unnecessary. Although *O. virilis* significantly suppressed biomass of chironomids (Fig. 2.3), they did not exert impacts on other herbivores such as the Ephemeroptera (Fig. 2.3). It is possible that the negative effect of any crayfish predation on the ephemeropterans was offset by a release from large anisopteran predation in the presence of crayfish.

Effects on periphyton

The direct negative impact of crayfish on periphyton biomass likely involved the combined effects of grazing and physical disturbance while this omnivore foraged for invertebrate prey, but dominated by active *O. virilis* grazing. Lodge et al. (1994) used the proportion of chl *a*/dry biomass of periphyton as a proxy for the impact *O. rusticus* had on the periphyton matrix; concluding that because there was a significant difference in chl *a*/ dry mass (Fig. 9 in Lodge et al. 1994), but no significant difference in chl *a*. *O. rusticus* was likely physically disturbing the loosely attached detrital components of the periphyton matrix, but not the attached algal component represented by chl *a* (Lodge et al. 1994). Here I show the opposite response of the periphyton matrix to the presence of *O. virilis*, perhaps highlighting an interesting difference in the impact each of these congeners has. *O. virilis* in enclosures reduced the total chl *a* (Fig. 2.7), but had no effect on the relative proportion of chl *a* to dry mass periphyton (Fig. 2.6). From this evidence I propose that *O. virilis* is in fact grazing the periphyton matrix and not simply disturbing it with its foraging activity.

Further, gut analysis revealed that crayfish consumed periphytic detritus, which contained bacteria and many of the algal groups that were detected using HPLC analysis. Therefore, crayfish suppressed algal pigments partly by direct consumption of loosely-attached species. These findings agree well with the findings of Keller et al. (1999) who found significantly higher periphyton biomass in recovering acidified lakes of northeastern Ontario in which crayfish had not yet become re-established. However, firmly-attached filamentous green algae (chlorophyll *b*) increased steadily in the presence of crayfish, indicating the relative inefficiency of crayfish as grazers when compared with other benthic invertebrates, such as ephemeropterans and trichopterans (Steinman 1996).

Unlike the herbivorous zoobenthos, omnivorous crayfish exhibited a more enriched $\delta^{13}C$ signal relative to periphyton (Fig. 2.8). Although the $\Delta\ \delta^{13}C$ may have been a result of preferential assimilation of certain components of organic matter, which do not reflect its bulk $\delta^{13}C$ signal (McCutchan et al. 2003), it is more likely that the length of this experiment was not great enough to allow for tissue turn over and detection of *O. virilis* diet in the enclosures (Stenroth et al. 2006). Thus the difference between crayfish and periphyton $\delta^{13}C$ signatures may reflect consumption of unmeasured alternate sources of organic carbon in the source lake (L468), such as $\delta^{13}C$ enriched epilithic algae (France and Peters 1995). However, *O. virilis* were observed non-selectively consuming periphyton during the experiment (ID Phillips, personal observation), and sources of macrophytes or detritus outside the leaf packs were sparse.

Table 2.1.a. Pooled taxa list of invertebrates collected by abundance, biomass and treatment. Trophic relationship codes: P = predator, C = collector, G = gatherer, F = filterer, S = shredder.

Taxa	Treatment				
	Abundance Crayfish (No. ind.)	Abundance Control (No. ind.)	Biomass Crayfish (mg)	Biomass Control (mg)	Trophic Relationship
Insecta					
Coleoptera					
Gyrinidae					
Gyrinus	72	104	7.20	11.50	P*
Dineutus	9	9	5.70	3.70	P*
Dytiscidae					
Laccophilus	1	0	0.10	0.00	P*
Diptera					
Chironomidae	2935	4500	29.35	49.56	C*
Ceratopogonidae	1	2	0.00	0.00	P*
Ephemeroptera					
Baetidae					
Procloeon	0	1	0.00	1.50	C, G*
Caenidae					
Caenis	142	115	63.94	51.50	C, G*
Heptageniidae					
Stenacron	5	5	7.60	5.60	C, G*
Tricorythidae					
Tricorythodes	8	7	12.86	10.98	C, G*
Hemiptera					
Corixidae	3	5	0.27	3.70	P*
Trichoptera					
Polycentropodidae					
Polycentropus	13	10	5.18	5.60	P, C, F, S*
Paranyctiophylax	2	4	2.07	2.37	P, C, F, S*
Nyctiophylax	4	5	2.40	5.40	P*
Hydroptilidae					
Oxyethira	6	3	0.30	0.19	C, G*
Leptoceridae					
Oecetis	63	84	58.08	73.00	P, S*
Nectopsyche	1	0	1.60	0.00	P, C, S, G*
Odonata					
Aeshnidae					
Aeshna	45	67	160.53	895.86	P*
Corduliidae					
Somatochlora	9	9	20.74	11.95	P*
Cordulia	11	16	25.98	83.79	P*
Macromia	0	1	0.00	6.00	P*

Table 2.1.b. Table 2.1.a continued

Taxa	Treatment Abundance Crayfish (No. ind.)	Abundance Control (No. ind.)	Biomass Crayfish (mg)	Biomass Control (mg)	Trophic Relationship
Gomphidae					
Gomphus	1	2	0.44	1706.61	P*
Hagenius	0	1	0.00	4.90	P*
Libellulidae					
Miathyria	1	0	0.80	0.00	P*
Sympetrum	14	11	29.54	128.38	P*
Leucorrhinia	0	2	0.00	32.42	P*
Coenagrionidae					
Enallagma	14	6	36.96	10.95	P*
Clitellata					
Gnathobdellida					
Hirudinidae					
Percymoorensis marmorata (Say)	2	0	975.50	0.00	P†
Pharyngobdellida					
Erpobdellidae					
Erpobdella punctata (Leidy)	3	8	0.18	105.54	P‡
Nephelopsis obscura (Verrill)	0	5	0.00	223.50	P¥, £
Glossiphoniidae					
Placobdella	9	5	56.40	33.60	P†
Helobdella stagnalis (Linnaeus)	1	1	0.02	0.02	P§
Pelecypoda					
Veneroida					
Sphaeriidae	0	2	0.00	0.04	F†
Gastropoda					
Basommatophora					
Ancylidae					
Ferrissia rivularis (Say)	1	0	0.02	0.00	G†
Total	3376	4989	1503.76	3468.16	

Source literature:

*	Merritt and Cummins 1996	¥	Anholt 1986
†	Clifford 1991	£	Davies et al. 1996
‡	Barton and Metcalfe 1985	§	Bradley and Reynolds 1987

Table 2.2. Repeated Measures ANOVA results for the negative effects of crayfish introductions on periphyton and benthic invertebrate biomasses. Values are *F* statistics for n=5 replicates. $*p<0.05$, $**p<0.001$.

Source	Treatment	Time	Treatment X Time
Periphyton	117.72**	903.2**	13.918**
Total Invertebrates	5.66*	1.58	0.81
Chironomidae (Diptera)	14.90**	14.97**	2.27
Caenis (Ephemeroptera)	0.489	26.64**	0.96
Oecetis (Trichoptera)	0.269	3.83*	0.548
Aeshna (Anisoptera)	11.49**	5.6**	2.6
Enallagma (Zygoptera)	6.35*	2.99	0.63
Hirudinea	11.79**	0.94	0.94

Table 2.3. Repeated Measures ANOVA results showing the negative effects of crayfish introductions on pigment concentrations. Values are F statistics for n=5 replicates. *p<0.05, **p<0.001.

Source	Treatment	Time	Treatment X Time
Fucoxanthin	69.43**	2.38	2.013
Diatoxanthin	49.00**	13.54**	1.36
Lutein/Zeaxanthin	31.63**	4.84**	2.36
Chlorophyll *a*	59.60**	3.88*	3.65*
Chlorophyll *b*	44.46**	9.74**	3.709*

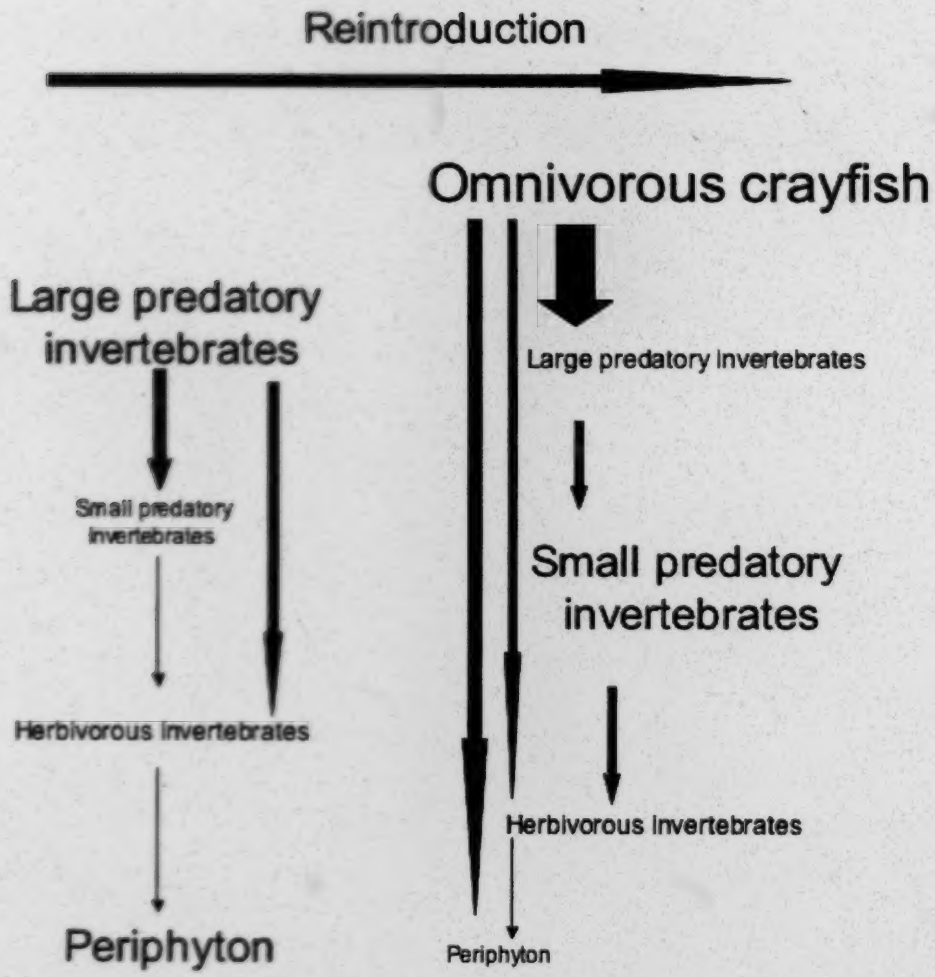

Figure 2.1. Hypothesized direct and indirect effects of omnivorous *Orconectes virilis* on periphytic algae upon reintroduction to post-acidification lakes. In post-acidification lakes (left) devoid of the natural *O. virilis* population, large predatory invertebrates control the abundance of other invertebrate predators, and herbivorous invertebrates to release periphyton from grazing pressure. The reintroduction of extirpated *O. virilis*, will result in significant top-down suppression of large invertebrate predators, however this will not translate into a trophic-cascade as *O. virilis* will exert direct predation on smaller invertebrates and grazing pressure on periphyton as well.

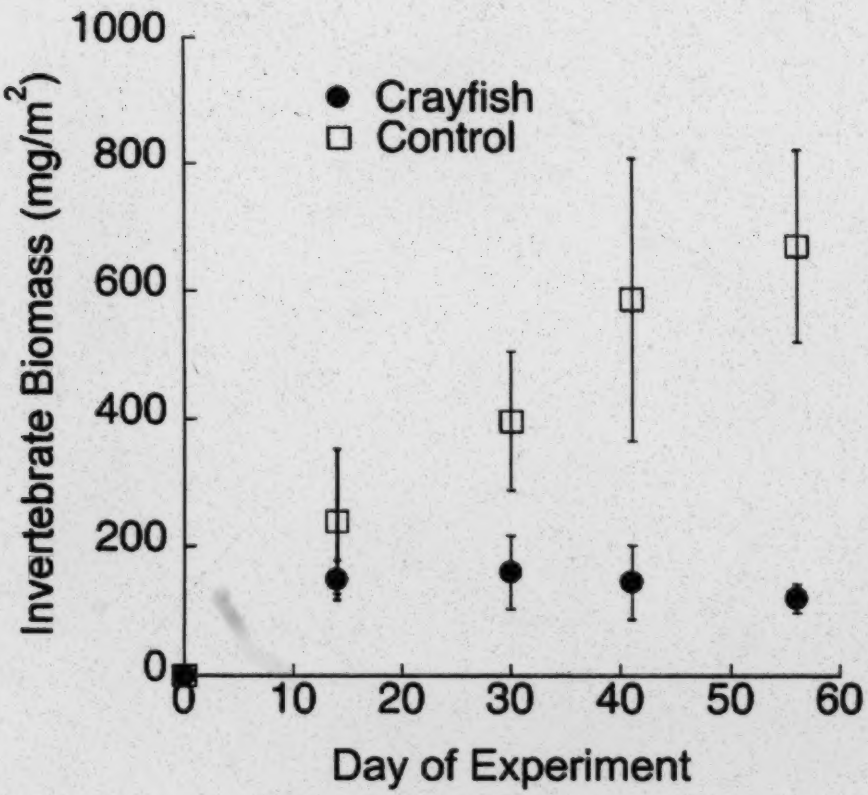

Figure 2.2. Mean (±1 SE) biomass of total benthic invertebrates in *O. virilis* enclosures, and control enclosures in L302S from June to August 2004.

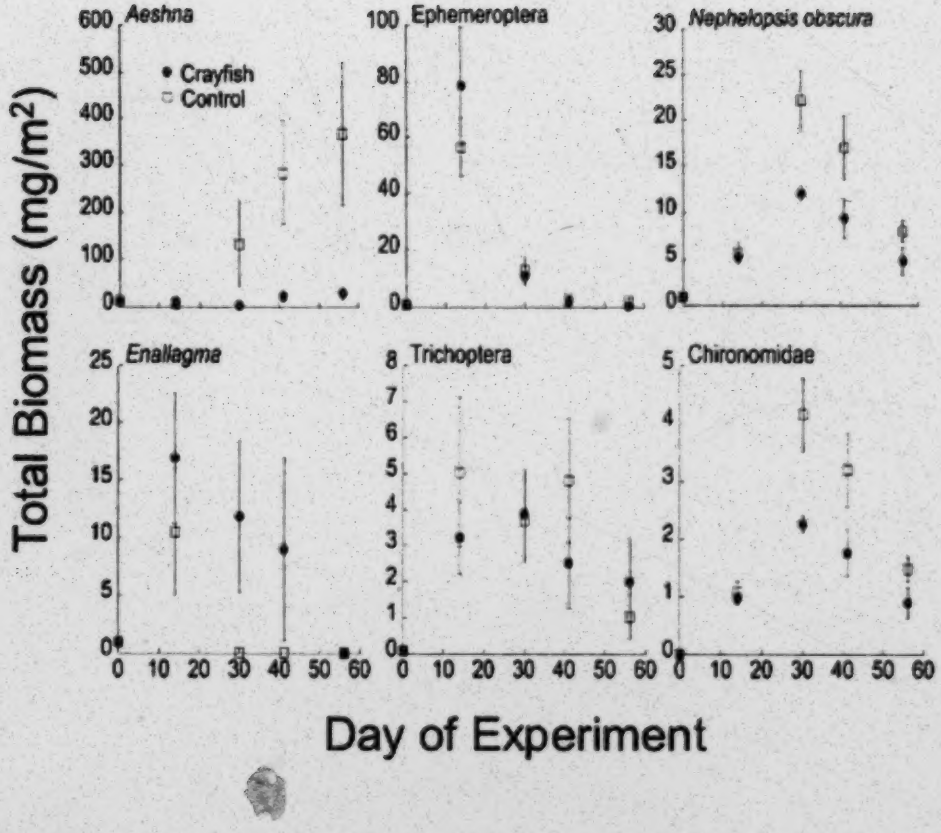

Figure 2.3. Mean (±1 SE) biomass of benthic invertebrates in *O. virilis* enclosures, and control enclosures in L302S from June to August 2004.

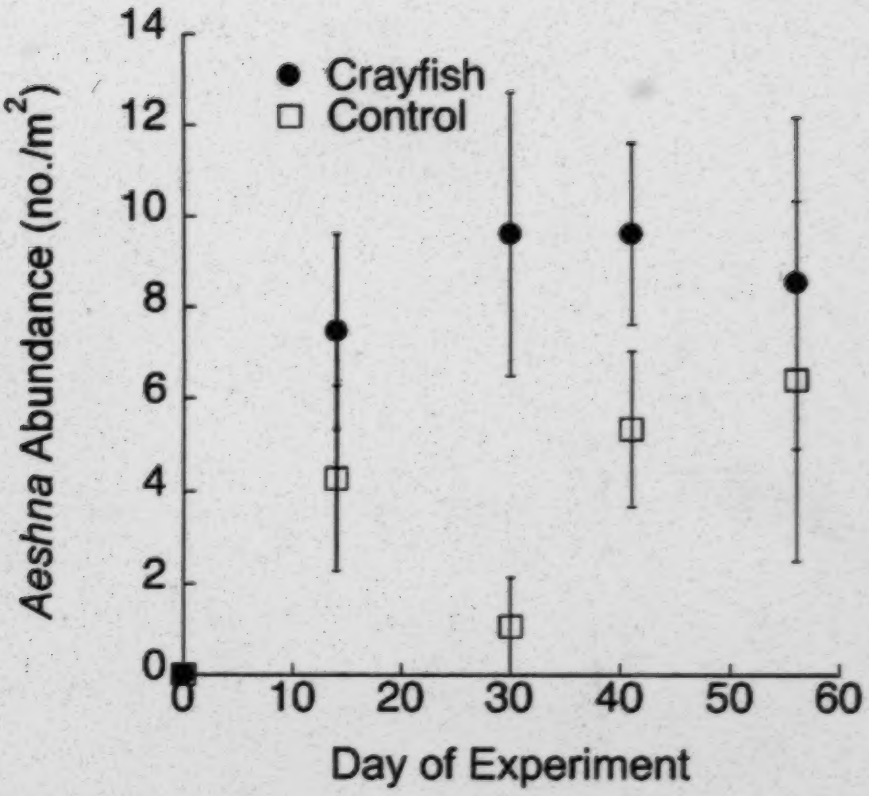

Figure 2.4. Mean (±1 SE) abundance of the dominant benthic predator *Aeshna* in *O. virilis* enclosures, and control enclosures in L302S from June to August 2004.

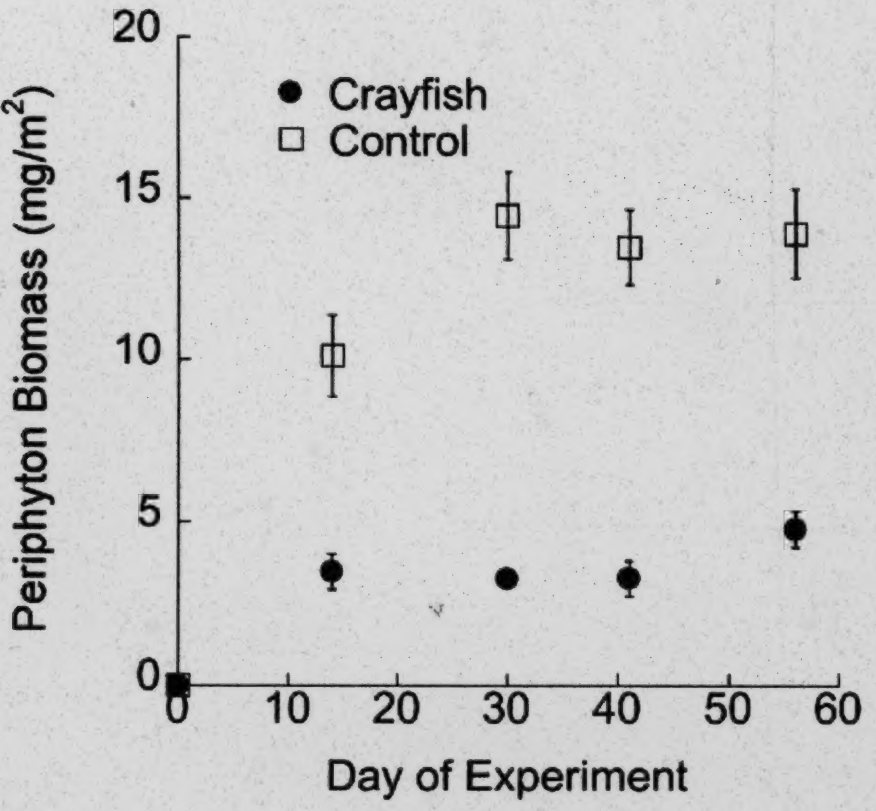

Figure 2.5. Mean (±1 SE) biomass of periphyton in *O. virilis* enclosures, and control enclosures in L302S from June to August 2004.

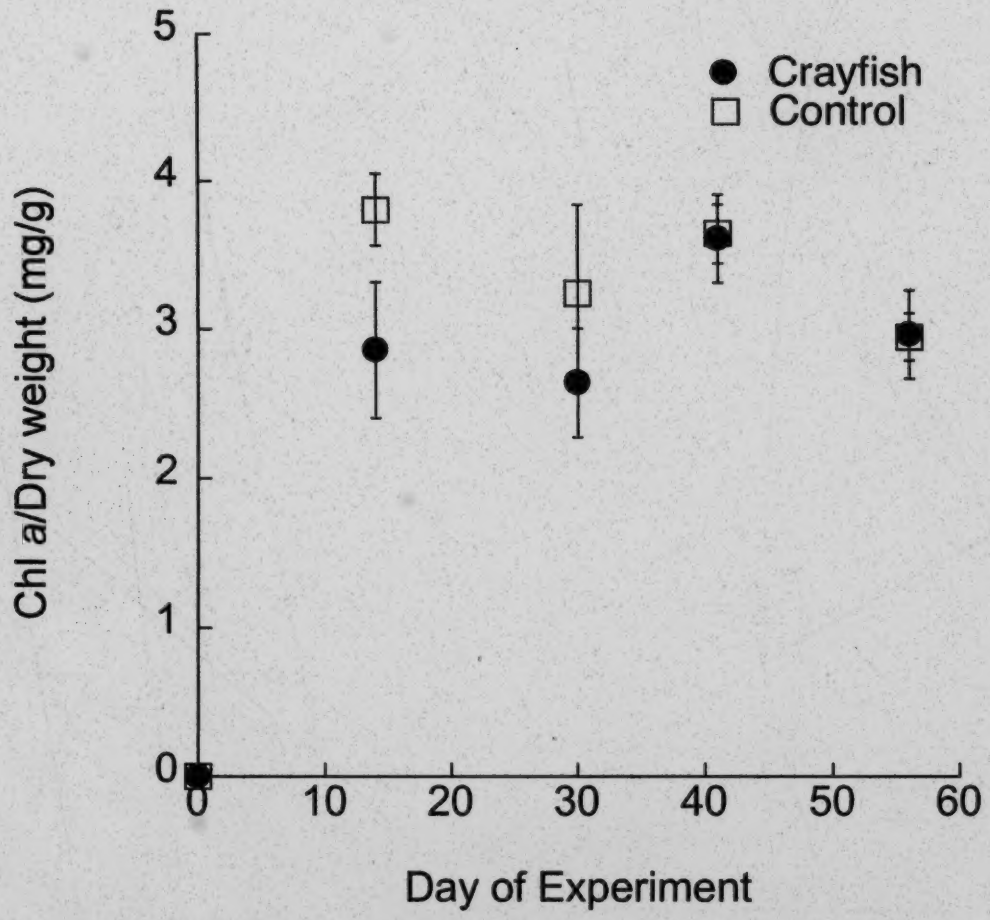

Figure 2.6. Mean (±1 SE) chl *a* pigment concentration per unit dry weight from tiles in *O. virilis* enclosures, and control enclosures in L302S from June to August 2004.

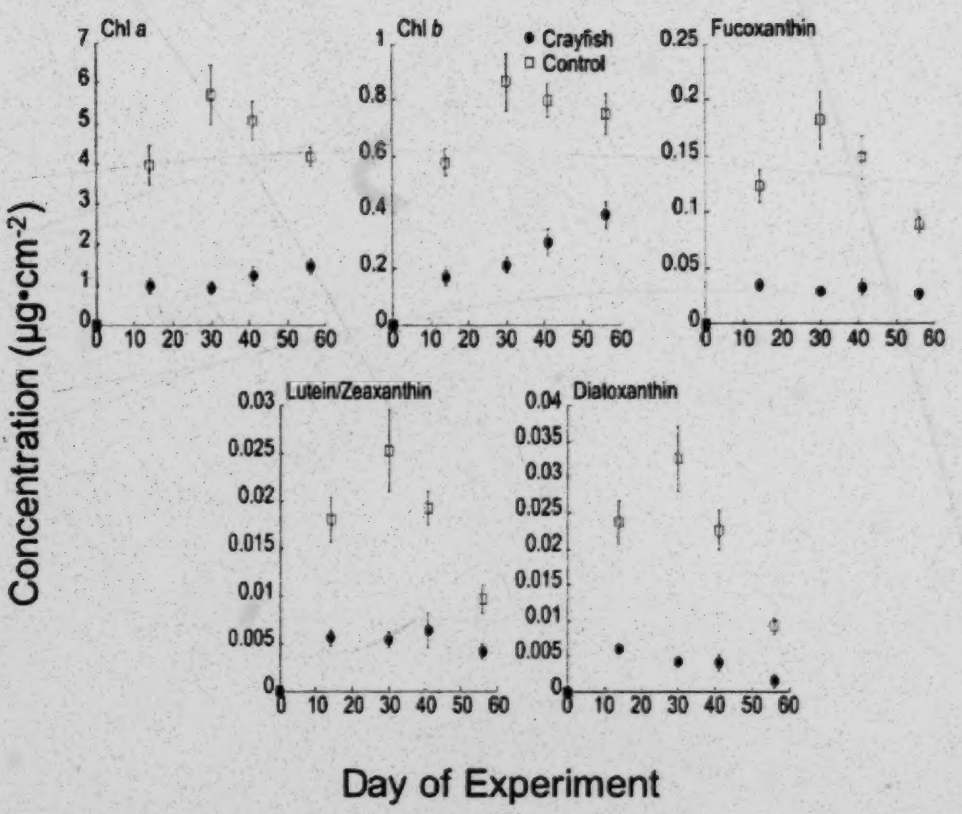

Figure 2.7. Mean (±1 SE) concentration of algal pigments in *O. virilis* enclosures, and control enclosures in L302S from June to August 2004.

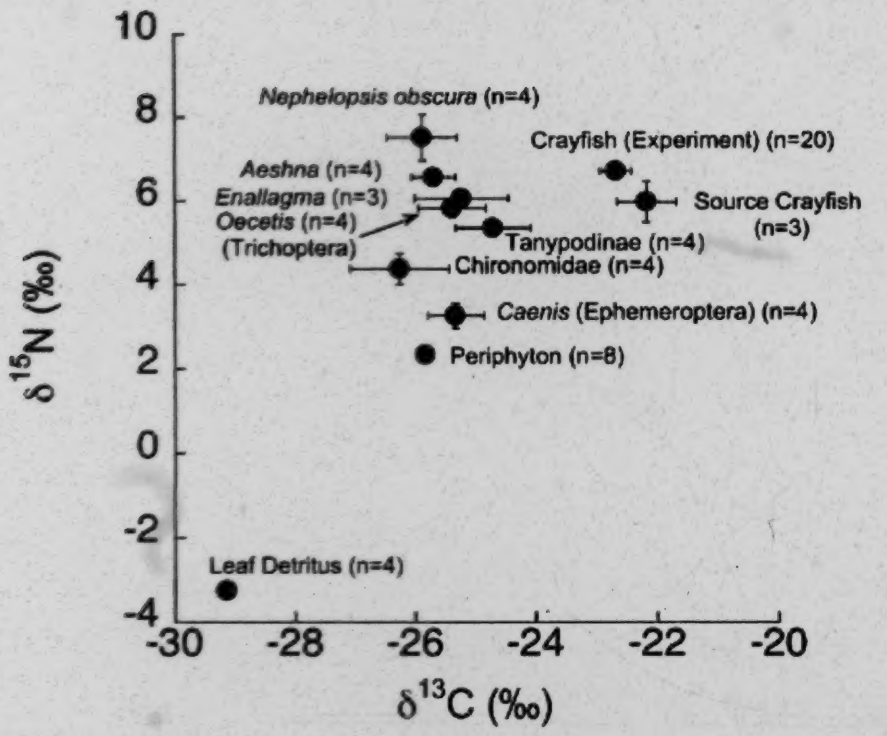

Figure 2.8. Results of stable isotope analyses showing mean (±1 SE) $\delta^{15}N$ and $\delta^{13}C$ of benthic fauna from L302S over summer 2004. Replicates used in analysis in parentheses.

LITERATURE CITED

Anholt, B. 1986. Prey selection by the predatory leech *Nephelopsis obscura* in relation to three alternative models of foraging. Canadian Journal of Zoology **64**:649-655.

Barton, D. R., and J. L. Metcalfe. 1986. Life cycles, reproduction, and diets of *Dina dubia* and *Erpobdella punctata* (Hirudinea: Erpobdellidae) in Canagagigue Creek, Ontario. Canadian Journal of Zoology **64**:640-648.

Benke, A. C., P. H. Crowley, and D. M. Johnson. 1982. Interactions among coexisting larval Odonata: an in situ experiment using small enclosures. Hydrobiologia **94**:121-130.

Benke, A. C., A. D. Huryn, L. A. Smock, and J. B. Wallace. 1999. Length-mass relationships for freshwater macroinvertebrates in North America with reference to the southeastern United States. Journal of the North American Benthological Society **18**:308-343.

Bohonak, A. J., and D. G. Jenkins. 2003. Ecological and evolutionary significance of dispersal by freshwater invertebrates. Ecology Letters **6**:783-796.

Bradley, M. D. K., and J. D. Reynolds. 1987. Diet of the leeches *Erpobdella octoculata* (L) and *Helobdella stagnalis* (L) in a lotic habitat subject to organic

pollution. Freshwater Biology **18**:267-275.

Brönmark, C. 1994. Effects of tench and perch on interactions in a freshwater benthic food chain. Ecology **75**:1818-1828.

Brönmark, C., S. P. Klosiewski, and R. A. Stein. 1992. Indirect effects of predation in a freshwater benthic food chain. Ecology **73**:1662-1674.

Carbone, J., W. Keller, and R. W. Griffiths. 1998. Effects of changes in acidity on aquatic insects in rocky littoral habitats of lakes near Sudbury, Ontario. Restoration Ecology **6**:376-389.

Charlebois, B. S., and G. A. Lamberti. 1996. Invading crayfish in a Michigan stream: direct and indirect effects on periphyton and macroinvertebrates. Journal of the North American Benthological Society **15**:551-563.

Clifford, H. F. 1991. Aquatic Invertebrates of Alberta. The University of Alberta Press, Edmonton, Alberta, Can.

Davies, I. J. 1989. Population collapse of the crayfish *Orconectes virilis* in response to experimental whole-lake acidification. Canadian Journal of Fisheries and Aquatic Sciences **46**:910-922.

Davies, R. W. 1971. A key to the freshwater Hirudinoidea of Canada. Journal of the Fisheries Research Board of Canada **28**:543-552.

Davies, R. W., E. Dratnal, and L. R. Linton. 1996. Activity and foraging behaviour in the predatory leech *Nephelopsis obscura* (Erpobdellidae). Functional Ecology **10**:51-54.

DeNiro, M. J., S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta **42**:495-506.

Dionne, M. and C. L. Folt. 1991. An experimental analysis of macrophyte growth forms as fish foraging habitat. Canadian Journal of Fisheries and Aquatic Sciences **48**: 123-131.

Dorn, N. J., and G. G. Mittelbach. 2004. Effects of a native crayfish (*Orconectes virilis*) on the reproductive success and nesting behavior of sunfish (*Lepomis* spp.). Canadian Journal of Fisheries and Aquatic Sciences **61**:2135-2143.

Dorn, N. J., and J. M. Wojdak. 2004. The role of omnivorous crayfish in littoral communities. Oecologia **140**:150-159.

France, R. L. 1993. Influence of lake pH on the distribution, abundance and health of crayfish in Canadian Shield lakes. Hydrobiologia **271**:65-70.

France, R. L., and N. C. Collins. 1993. Extirpation of crayfish in a lake affected by long-range anthropogenic acidification. Conservation Biology **7**:184-188.

Gunn, J. M., and W. Keller. 1990. Biological recovery of an acid lake following reductions in industrial emissions of sulphur. Nature **345**:431-433.

Hanson, J. M., P. A. Chambers, and E. E. Prepas. 1990. Selective foraging by the crayfish *Orconectes virilis* and its impact on the macroinvertebrates. Freshwater Biology **24**:481-491.

Hecky, R. E., and R. H. Hesslein. 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. Journal of the North American Benthological Society **14**:631-653.

Helms, B. S., and R. P. Creed. 2005. The effects of 2 coexisting crayfish on an Appalachian river community. Journal of the North American Benthological Society **24**:113-122.

Heneberry, J. H., K. Errulat, M. Robataille, L. Whitmore, and J. R. M. Morris. 1992. A preliminary investigation on the distribution of freshwater crayfish (Decapoda: Cambaridae) in 32 lakes of the Sudbury Region of Northeastern Ontario. Project Report, Laurentian University, Sudbury, Ontario, Canada. 58

Pages.

Hilsenhoff, W. L. 1995. Aquatic Insects of Wisconsin: Keys to Wisconsin Genera and Notes on Biology, Habitat, Distribution and Species. Natural History Museums Council, Madison.

Hobson, K. S., and H. E. Welch. 1992. Determination of trophic relationships in a high arctic food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Marine Ecology Progress Series **84**:9-18.

Hogsden, K. L., and R. D. Vinebrooke. 2005. Environmental predictors of benthic consumers and autotrophic communities along a recovery gradient. Canadian Journal of Fisheries and Aquatic Sciences **62**:2226-2239.

Johnson, D. M. 1991. Behavioral ecology of larval dragonflies and damselflies. Trends in Ecology and Evolution **6**:8-14.

Jones, C. G., J. H. Lawton, M. Shachak. 1994. Organisms as ecosystem engineers. Oikos **69**: 373-386.

Keller, W., J. M. Gunn, and N. D. Yan. 1992. Evidence for biological recovery in acid-stressed lakes near Sudbury, Canada. Environmental Pollution **78**:79-85.

Keller, W., J. H. Heneberry, J. M. Gunn. 1999. Effects of emission reductions from the Sudbury smelters on the recovery of acid- and metal-damaged lakes. Journal of Aquatic Ecosystem Stress and Recovery **6**:189-198.

Kirk, D. J., and L. A. Smock. 2000. Interspecific and intraspecific interactions between crayfish (*Cambarus longulus*) and hellgrammites (*Corydalus cornutus*) and the influence of a predatory fish (*Micropterus dolomieu*). American Midland Naturalist **144**:317-327.

Kling, G. W., B. Fry, and W. J. O'Brian. 1992. Stable isotopes and structure in arctic lakes. Ecology **73**:561-566.

Lodge, D. M., M. W. Kershner, J. E. Aloi, and A. P. Covich. 1994. Effects of an omnivorous crayfish (*Orconectes rusticus*) on a freshwater littoral food web. Ecology **75**:1265-1281.

McCarthy, J. M., C. L. Hein, J. D. Olden, and M. J. Vander Zanden. 2006. Coupling long-term studies with meta-analysis to investigate impacts of non-native crayfish on zoobenthic communities. Freshwater Biology **51**: 224-235.

McCutchan, J. H., Jr., W. M. Lewis, Jr., C. Kendall, and C. C. McGrath. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos **102**:378-390.

McNaught, A. S., D. W. Schindler, B. R. Parker, A. J. Paul, R. S. Anderson, D. B. Donald, and M. Agbeti. 1999. Restoration of the food web of an alpine lake following fish stocking. Limnology and Oceanography **44**:127-136.

Menge, B. A., and J. P. Sutherland. 1987. Community regulation: variation in disturbance, competition, and predation in relation to environmental stress and recruitment. The American Naturalist **130**:730-757.

Merritt, R. W. and K. W. Cummins. 1996. An Introduction to the Aquatic Insects of North America. 3rd Edition. Kendall/Hunt Publishing Company, Dubuque, Iowa, USA.

Minagawa, M., and E. Wada. 1984. Stepwise enrichment of $\delta^{15}N$ along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochimica et Cosmochimica Acta **48**:1135-1140.

Momot, W. T., and H. Gowing. 1977. Production and population dynamics of the crayfish *Orconectes virilis* in three Michigan lakes. Journal of the Fisheries Research Board of Canada **34**:2041-2055.

Morin, P. J. 1984. Odonate guild composition: experiments with colonization history and fish predation. Ecology **65**:1866-1873.

Nyström, P., C. Brönmark, and W. Granéli. 1996. Patterns in benthic food webs: a role for omnivorous crayfish? Freshwater Biology **36**:631-646.

Nyström, P., C. Brönmark, and W. Granéli. 1999. Influence of an exotic and a native crayfish species on a littoral benthic community. Oikos **85**:545-553.

Olsen, T. M., D. M. Lodge, G. M. Capelli, and R. J. Houlihan. 1991. Mechanisms of impact of an introduced crayfish (*Orconectes rusticus*) on littoral congeners, snails, and macrophytes. Canadian Journal of Fisheries and Aquatic Sciences **48**: 1836-1846.

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics **18**:293-320.

Polis, G. A., and R. D. Holt. 1992. Intraguild predation: the dynamics of complex trophic interactions. Trends in Ecology and Evolution **7**:151-154.

Power, M. E. 1990. Effects of fish in river food webs. Science **250**:811-814.

Power, M. E. 1992. Habitat heterogeneity and the functional significance of fish in river food webs. Ecology **73**:1675-1688.

Power, M. E., D. Tilman, J. A. Estes, B. A. Menge, W. J. Bond, L. S. Mills, G. Daily, J. C. Castilla, J. Lubchenco, R. T. Paine. 1996. Challenges in the quest for keystones. Bioscience **46** 609-620.

Rosenfeld, J. S., and J. C. Roff. 1992. Examination of the carbon base in southern Ontario streams using stable isotopes. Journal of the North American Benthological Society **11**:1-10.

Scheffer, M., S. Carpenter, J. A. Foley, C. Folke, B. Walker. 2001. Catastrophic shifts in ecosystems. Nature **413**: 591-596.

Schindler, D. W., T. M. Frost, K. H. Mills, P. S. S. Chang, I. J. Davies, L. Findlay, D. F. Malley, J. A. Shearer, M. A. Turner, P. J. Garrison, C. J. Watras, K. Webster, J. M. Gunn, P. L. Brezonik, and W. A. Swenson. 1991. Comparisons between experimentally- and atmospherically-acidified lakes during stress and recovery. Proceedings of the Royal Society of Edinburgh **97B**: 193-226.

Smith, K. G., J. D. Clark. 1994. Black bears in Arkansas: characteristics of a successful translocation. Journal of Mammalogy **75**: 309-320.

Souty-Grosset, C. 2005. Introduction: The EU-network CRAYNET-impacts on fundamental questions. Bulletin Français de la Pêche et de la Pisciculture **367-377**:495-503.

Steinman, A. D. 1996. Effects of grazers on freshwater benthic algae. Pages 341-373 *in* R. J. Stevenson, M. L. Bothwell, and R. L. Lowe, editors. Algal ecology: freshwater benthic ecosystems. Academic Press, San Diego, California, USA.

Stenroth, P., N. Homqvist, P. Nyström, O. Berglund, P. Larsson, and W. Granéli. 2006. Stable isotopes as an indicator of diet in omnivorous crayfish (*Pacifastacus leniusculus*): the influence of tissue, sample treatment, and season. Canadian Journal of Fisheries and Aquatic Sciences **63**:821-831.

Stoks, R., and M. A. McPeek. 2003*a*. Predators and life histories shape *Lestes* damselfly assemblages along a freshwater habitat gradient. Ecology **84**:1576-1587.

Stoks, R., and M. A. McPeek. 2003*b*. Antipredator behavior and physiology determine *Lestes* species turnover along the pond-permanence gradient. Ecology **84**:3327-3338.

Taugbøl, T., and S. Peay. 2004. Roundtable session 3: Reintroduction of native crayfish and habitat restoration. Bulletin Français de la Pêche et de la Pisciculture **372-373**:465-471.

Turner, M. A., D. W. Schindler, D. L. Findlay, M. B. Jackson, and G. G. C. Robinson. 1995*a*. Distribution of littoral algal associations by experimental lake acidification. Canadian Journal of Fisheries and Aquatic Sciences **52**:2238-2250.

Turner, M. A., G. G. C. Robinson, B. E. Townsend, B. J. Hann, J. A. Amaral. 1995*b*. Ecological effects of blooms of filamentous green algae in the littoral zone of an acid lake. Canadian Journal of Fisheries and Aquatic Sciences **52**:2264-2275.

Varley, N., and M. S. Boyce. 2006. Adaptive management for reintroductions: updating a wolf recovery model for Yellowstone National Park. Ecological Modelling **193**:315-339.

Vinebrooke, R. D., and P. R. Leavitt. 1999. Phytobenthos and phytoplankton as potential indicators of climate change in mountain lakes and ponds: a HPLC-based pigment approach. Journal of the North American Benthological Society **18**: 15-33.

Vinebrooke, R. D., Turner, M. A., Kidd, K. A., Hann, B. J., and Schindler, D. W. 2001. Truncated foodweb effects of omnivorous minnows in a recovering acidified lake. Journal of the North American Benthological Society **20**:629-642.

Whitledge, G. W., and C. F. Rabeni. 1997. Energy sources and ecological role of crayfishes in an Ozark stream: insights from stable isotopes and gut analysis. Canadian Journal of Fisheries and Aquatic Sciences **54**:2555-2563.

Wiggins, G. B. 1996. Larvae of the North American caddisfly genera 2nd edition. University of Toronto Press, Toronto, ON.

CHAPTER THREE

GENERAL CONCLUSIONS AND MANAGEMENT IMPLICATIONS

GENERAL CONTEXT

In my thesis I reviewed the current understanding of the range distributions, areas of potential range expansion, and the impact of the crayfish *Orconectes virilis* and *O. rusticus* on the ecosystems they inhabit. I then tested the impact that re-introduction of *O. virilis* would have on the benthic ecosystem of post-acidification boreal lakes in Canada. In concert with past documentation of crayfish diet and the hypotheses developed for this experiment, *O. virilis* demonstrated a strongly omnivorous effect on the benthic communities of L302S. However, *O. virilis* also had unanticipated indirect effects on the composition of the benthic invertebrate community. Further, the degree to which *O. virilis* can affect the productive capacity of these lakes, demonstrated here to be 70 % invertebrate and 90 % periphyton reduction, was not appreciated in the development of my original hypotheses. If reintroductions or invasions of crayfish occur in ecosystems that lack predators of orconectids, these organisms may function as trophic cul-de-sacs that reduce the transfer of energy from lower trophic levels to forage fish and other organisms.

CONCLUSIONS

Areas of future research

Much remains to be studied about the potential effects that orconectid

crayfish may have on pelagic communities. Research by Dorn and Wojdak (2004) has identified that *O. virilis* has the potential to indirectly affect plankton communities, at least in ponds. This possibility has received little attention, and is important to further explore.

In addition, Mosindy and Rusak (1991) have found that crayfish can form up to 70% of the diet of lake sturgeon (*Acipenser fluvescens* Rafinesque) (a species at risk) in the Rainy River, Ontario. Therefore the consequences of dietary shift from *O. virilis* to *O. rusticus* for sturgeon populations may be an important consideration in the management of this species at risk.

Further, in order to appreciate the effectiveness of crayfish control by fish it is necessary to understand how body size dynamics in fish populations play a role in constraining invasions. It is possible that the reduced size of fish populations in Alberta may have facilitated *O. virilis* invasion. Research on the ability of fish populations with a preponderance of large individuals to check *O. virilis* and *O. rusticus* range expansion is necessary. Canadian locations for research where size distributions could be manipulated include areas where *O. rusticus* has already become established in northwestern Ontario, particularly in the Lake of the Woods watershed.

Monitoring of range expansion

O. rusticus is a prolific invader that quickly colonizes new water bodies and displaces native congeners (Capelli 1982). It will likely expand its current range throughout the Canadian watersheds that are adjacent to its current range. Without

stringent controls on the handling and transport of these species in Canada, orconectid crayfish may be transported easily to lakes and rivers outside their current range. Angling activity has been implicated as the primary mechanism of orconectid introductions across watersheds (Page 1985).

Ironically, the best method of monitoring the range expansions of both species likely lies with sport fishermen. Educating fishermen about species identification and the negative ecological impacts that such introductions can have, will reduce future introductions and warn us of invasion progress. There are far fewer aquatic invertebrate ecologists than there are anglers, and with a little education, the taxonomic skills of anglers could contribute to monitor range changes of *O. virilis* and *O. rusticus*. Further, both groups have a vested interest in maintaining the ecological integrity of Canadian water bodies for the future, and cooperation is crucial to achieve this mutual goal.

Regions of likely range expansion

O. virilis is expanding its range in Alberta, but the areas invaded have yet to be documented let alone mapped. Therefore it is difficult to identify what impact the crayfish may be having, or what areas they may invade next. However, as *O. rusticus* is now present in northwestern Ontario it will likely spread through connecting water bodies, and expand its range further into Ontario and western Canada if left unchecked (Fig. 1.2). Momot (1992) identifies the warm shallow bays from Pigeon Bay in Lake Superior east to Marathon, Ontario, as a Canadian area of concern. More research is necessary in southern Ontario to determine the extent of

the *O. rusticus* invasion occurring there today, and to identify methods of preventing its proliferation to adjoining watersheds.

If bait-bucket introductions continue without an attitudinal change by anglers in the use of crayfish as bait, *O. virilis* and *O. rusticus* will likely continue to proliferate outside their current range no matter what measures are taken to contain them. Legislation prohibiting possession of rusty crayfish has been enacted in Illinois and Wisconsin (Taylor et al. 1996) because of its aggressive behavior, its ability to displace native species, and its extreme ecological impact (Capelli 1982, Page 1985, Lodge et al. 1994). Such regulations are needed in Canada; recently Manitoba has identified *O. rusticus* as a prohibited species. The negative impacts of bait-bucket releases also need to be emphasized to anglers who may otherwise be unaware of the damage non-indigenous crayfish introductions can have.

Management implications of reintroduction

Reintroduction has been clarified by the International Union for the Conservation of Nature (IUCN) Reintroduction Specialist Group as a key strategy for re-establishing extirpated species to their native home range (IUCN 1998). Species re-introductions are often performed in terrestrial ecosystems (see Clark et al. 2002), and the results have been somewhat promising. Although such reintroductions have been costly, time consuming, and only ~11% successful (Earnhardt 1999), the recommendations arising from these experiments appear compatible with the nature of crayfish populations. For example, reintroduction success is enhanced with large founding populations, low environmental variation,

high rate of population increase, and low intraspecific competition (Griffith et al. 1989). Fortunately crayfish reintroductions in boreal ecosystems would benefit from large source populations of *O. virilis* being readily available from surrounding areas. Further, with pH returned to suitable levels for *O. virilis* inhabitation and reproduction (>5.9; France 1993) re-colonization will likely be rapid. Lake ecosystems lack the dramatic environmental variation experienced by terrestrial ecosystems and female *O. virilis* can theoretically produce >250 offspring each year (Corey 1987). Therefore, as these crayfish will be entering a lake devoid of conspecifics or resident crayfish they will be able to proliferate and quickly re-establish.

Native European crayfish populations have experienced range restrictions, and local crayfish extirpations in the past primarily due to the crayfish plague (*Aphanomyces astaci*) and plague-carrying crayfish species (Taugbøl and Skrudal 1999, Vogt 1999). Organizations such as the European-network CRAYNET are discussing reintroduction of indigenous crayfish and habitat restoration as methods to restore populations recently lost, extend the distribution of indigenous crayfish into their historic range, or to create isolated reserves of crayfish for purposes of genetic diversity preservation (Souty-Grosset 2005). Managers in Norway have already successfully reintroduced noble crayfish (*Astacus astacus* Linnaeus) to the Glomma and Halden watercourses, and guidelines for future reintroductions are being developed (Taugbøl and Peay 2004).

In North America, native crayfish reintroduction to post-acidification water-bodies lacking a native crayfish population such as *O. virilis* may be necessary to

return such ecosystems to pre-acidification condition. As long-range acid deposition affects whole watersheds, it is likely that significant barriers exist and restrict native populations of *O. virilis* from returning to their native range. However, *O. virilis* is not a species that is in danger of extinction, as its range and population is much larger than that of European crayfish. Further, the necessity of reintroduction in Europe has different biodiversity and socioeconomic consequences than here in North America. Native crayfish diversity has been seriously compromised by the introduction of plague spreading North American crayfish, and one of the major drivers of crayfish conservation is their importance in human diet throughout Europe (Swahn 2004).

Re-stocking of recovering acidified lakes with native crayfish in the absence of large benthivores will likely suppress energy transfer to higher trophic levels, thereby decoupling benthic from pelagic habitats. However, in the absence of a predator capable of consuming *O. virilis*, reintroduction of *O. virilis* may exert invader-like characteristics exerting detrimental effects on its environment. Mack et al. (2000) assert that a variety of characteristics can be used to describe invaders and the impact they express. If the mere presence of a new species alters fundamental ecological properties such as the dominant species in a community and the physical features of an ecosystem, plant productivity and nutrient cycling, then a non-indigenous species can be considered detrimental. I have shown that *O. virilis* has a dramatic effect on the benthic environment by replacing top benthic predators (leeches and odonates) (Fig. 2.3, 2.8), reducing periphyton biomass (Fig. 2.5), and total benthic algal productivity (Fig. 2.7). In addition, *O. virilis* may significantly

reduce the forage resources of fish populations, and may be a trophic dead-end seriously affecting nutrient cycling in lakes lacking significant crayfish predators such as smallmouth bass (*Micropterus dolomieu* Lacepede) (Roell and Orth 1993), rock bass (*Ambloplites rupestris* [Rafinesque]) (Roth and Kitchell 2005), large walleye (*Sander vitreus* [Mitchill]) (Roth and Kitchell 2005) and pike (*Esox lucius* [Linnaeus]) (Dorn and Mittelbach 1999). Therefore if aquatic managers are interested in returning aquatic ecosystems to their ancestral condition pre-acidification then it is certainly necessary to reintroduce a keystone species such as *O. virilis*. However, reintroduction would need be considered relative to the fish fauna present to avoid such detrimental effects and conflicting with commercial and sport fisheries of those species competing with *O. virilis* for benthic resources, but incapable of acting as *O. virilis* predators.

Although indirect food-web effects may be minor relative to the overall predator impact of either *O. virilis* or *O. rusticus*, it would be interesting to experimentally determine if such differences translate into larger cascading trophic effects. Such a difference would certainly be important to understand when modeling the impact of future *O. virilis* range expansions relative to that of *O. rusticus*.

LITERATURE CITED

Capelli, G. 1982. Displacement of northern Wisconsin crayfish by *Orconectes rusticus* (Gerard). Limnology and Oceanography **27**:741-745.

Clark, J. D., D. Huber, and C. Servheen. 2002. Bear reintroductions: lessons and challenges. Ursus **13**:153-163.

Corey, S, 1987. Comparative fecundity of four species of crayfish in southwestern Ontario, Canada (Decapoda, Astacidae). Crustaceana **52**:276-286.

Dorn, N. J., and G. G. Mittelbach. 1999. More than predator and prey: a review of interactions between fish and crayfish. Vie et Milieu **49**: 229-237.

Dorn, N. J., and J. M. Wojdak. 2004. The role of omnivorous crayfish in littoral communities. Oecologia **140**:150-159.

Earnhardt, J. M. 1999. Reintroduction programs: genetic trade-offs for populations. Animal Conservation **2**:279-286.

France, R. L. 1993. Influence of lake pH on the distribution, abundance and health of crayfish in Canadian Shield lakes. Hydrobiologia **271**:65-70.

Griffith, B., J. M. Scott, J. W. Carpenter, and C. Reed. 1989. Translocation as a species conservation tool: status and strategy. Science **245**:477-480.

International Union for the Conservation of Nature. 1998. Guidelines for re-

introductions. Prepared by the International Union for the Conservation of Nature Re-introduction Specialist Group. Gland, Switzerland and Cambridge, United Kingdom.

Lodge, D. M., M. W. Kershner, J. E. Aloi, and A. P. Covich. 1994. Effects of an omnivorous crayfish (*Orconectes rusticus*) on a freshwater littoral food web. Ecology **75**: 1265-1281.

Mack, R. N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout, and F. A. Bazzaz. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. Ecological Applications **10**:689-710.

Momot, W. T. 1992. Further range extensions of the crayfish *Orconectes rusticus* in the Lake Superior Basin of northwestern Ontario. The Canadian Field Naturalist **106**: 397-399.

Mosindy, T., and J. A. Rusak. 1991. An assessment of lake populations in Lake of the Woods and the Rainy River, 1987-1990. Ontario Ministry of Natural Resources. Lake of the Woods Fisheries Assessment Unit Tech. Rep. No. 1991-01, Queen's Printer for Ontario, Toronto, Canada.

Page, L. M. 1985. The crayfishes and shrimps (Decapoda) of Illinois. Illinois Natural History Survey Bulletin **33**: 335-448.

Roell, M. J., and D. J. Orth. 1993. Trophic basis of production of stream-dwelling smallmouth bass, rock bass, and flathead catfish in relation to invertebrate bait harvest. Transactions of the American Fisheries Society **122**: 46-62.

Roth, B. M., and J. F. Kitchell. 2005. The role of size-selective predation in the displacement of *Orconectes* crayfishes following rusty crayfish invasion. Crustaceana **78**: 297-310.

Souty-Grosset, C. 2005. Introduction: The EU-network CRAYNET-impacts on fundamental questions. Bulletin Français de la Pêche et de la Pisciculture **367-377**:495-503.

Swahn, J. O. 2004. The cultural history of crayfish. Bulletin Français de la Pêche et de la Pisciculture **372-373**:243-262.

Taugbøl, T., and S. Peay. 2004. Roundtable session 3: Reintroduction of native crayfish and habitat restoration. Bulletin Français de la Pêche et de la Pisciculture **372-373**:465-471.

Taugbøl, T., and J. Skrudal. 1999. The future of native crayfish in Europe – How to make the best of a bad situation? Crustacean Issues **11**:271-279.

Taylor, C. A., M. L. Warren, Jr., J. F. Fitzpatrick, Jr., H. H. Hobbs, III, R. F. Jezerinac, W. L. Pflieger, and H. W. Robinson. 1996. Conservation status of crayfishes of the United States and Canada. Fisheries **21**: 25-38.

Vogt, G. 1999. Diseases of European freshwater crayfish, with particular emphasis on interspecific transmission of pathogens. Crustacean Issues **11**:87-103.